IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Charlotte Moira Norfor Allerton, et al.	)	Serial No.: 10/727,168
Filed: 2 December 2003	)	Examiner: Grazier, Nyeemah
Attorney's Docket: PC25420 US	)	Group Art Unit: 1636
Title: Morpholine Dopamine Agonists	)	

DECLARATION OF DR. GILLIAN BURGESS

PURSUANT TO 37 CFR §1.132

I, Gillian Munro Burgess, hereby declare as follows:

1. I have a BSc (1st Class Honours) in pharmacology from the University of Glasgow and a PhD in Pharmacology from University College London and post-doctoral fellowships at the University of Paris XIe, at Medical College of Virginia, and NIEHS, Research Triangle Park, North Carolina.

2. Prior to my employment with Pfizer, I worked at Novartis as Laboratory Head in the Pain Unit. Since joining Pfizer in 1999, I have been Head of the Candidate Research Group (providing human pharmacology data and Safety Pharmacology data for the Pfizer Discovery portfolio), Head of the Urology Therapeutic Area and Research Therapeutic Area Head for the Gastrointestinal and Hepatology, and am now Research Therapeutic Area Head for Pain.

3. I am familiar with the subject-matter of the above application and the documents cited therein.

4. I am currently Research Therapeutic Area Head for the Pain Therapeutic Area and am responsible for a series of tests carried out on the compound of Example 67 of the present application (hereinafter 'Example 67') and 2-(6-aminopyrid-3-yl)-4-(1,1-dimethylethyl)morpholine, which is the compound of Example 3 of US patent 5077290, assigned to Merck & Co. Inc. (hereinafter 'Merck Example 3'), which is considered to represent the closest prior art. The protocol and results of these tests are presented in the attached Annex and Appendices.

5. The results of the tests showed that Example 67 demonstrated notably stronger activity at the D3 receptor in binding (2 different assay formats) and a functional assay, than the more active enantiomer (enantiomer 1) of Merck Example 3. Therefore, even stronger activity for Example 67 would be projected as compared to the racemic mixture as disclosed in Merck Example 3.

6. It is a general principle in the field of drug discovery that compounds of higher potency (binding and functional activity) are expected to elicit *in vivo* effects at lower unbound plasma concentrations with potential for a lower human dose size and reduced risk of adverse side effects. Therefore, given its enhanced potency at the D3 receptor, Example 67 would be expected to elicit a response at a lower dose and/or at unbound drug levels in the clinic than enantiomer 1 or, most particularly, the racemic mixture disclosed in Merck Example 3.

7. It is a commonly encountered feature of structure-activity relationships in the field of medicinal chemistry that small structural changes can have profound and unpredictable effects on biological activity, either advantageous or deleterious (several examples are described in: *Specific Substituent Effects*. C.G. Wermuth, Ed. Wermuth, C.G. Practice of Medicinal Chemistry (1996), 312-344. Publisher: Academic, Pub. London, UK – attached as Reference 1).

8. As a specific example, it is widely known that biological and pharmacological activity is often highly dependent upon stereochemistry. Several relevant examples are described in the review article *Stereoselectivity in drug action and disposition: an overview.* Patel, B. K.; Hutt, A. J. Ed. Reddy, I. K.; Mehvar, Reza. in: *Chirality in Drug Design and Development* **2004**, 139-190. Pub. Marcel Dekker, Inc., New York, N.Y – attached as Reference 2. Appreciation of stereochemical issues in drug design and development have increased in the last few decades, such that it is now deemed good practice, and a requirement of many pharmaceutical regulatory authorities, to develop chiral drugs as single enantiomers (and stereoisomers).

9. With particular relevance to this case, the structural changes and specific stereochemistry required to increase potency at the D3 receptor afforded by example 67 as compared with Merck Example 3, could not have been predicted and were not suggested by the prior art.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that wilful false statements and the like may jeopardize the validity of the above application or any patent granted thereon.

Declared at Sandwich, Kent, England this TENTH day of May 2007
By:

G. Burgess

Gillian Burgess

Before me:

Notary Public

Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

Annex

D3 Agonist Study Description and Results Summary

Agonist activity of compounds at the D3 receptor was measured using both binding and functional assays. The binding assay measures the affinity of the compound for the D3 receptor. The functional assay measures the ability of the agonist to produce a cellular response as a consequence of binding to the D3 receptor. Both are important measurements as sometimes compounds that bind to receptors do not induce cellular responses.

Example 3 of US patent 5077290 ('Merck Example 3') describes the compound 2-(6-amino-pyrid-3-yl)-4-(1,1-dimethylethyl)morpholine as the citrate salt as a racemic mixture of enantiomers. In order to generate unequivocal data in a functional assay format (where screening of racemic mixtures may generate uninterpretable results) the constituent enantiomers (referred to below as enantiomers 1 and 2 – enantiomer 1 being the first to elute from the chiral HPLC column referred to in Appendix 6) were prepared and tested as the free bases. Under the buffered conditions of the assay the salt form would not be expected to influence assay results.

Detailed test protocols are described as follows:

Appendix 1 - D3 binding assay 1 - Example 67 of the present application
Appendix 2 - D3 binding assay 1 - Merck Example 3, enantiomer 1
Appendix 3 - D3 binding assay 1 - Merck Example 3, enantiomer 2
Appendix 4 - D3 binding assay 2 - all tested compounds
Appendix 5 - D3 functional assay - all tested compounds

The data is summarised in the table below and in Figures 1 and 2.

Compound	**D3 binding assay 1 pK_i (K_i μM)**	**D3 binding assay 2 $pK_i \pm SD$ (K_i μM)**	**D3 functional agonist assay $pEC_{50} \pm SD$ (EC_{50} μM)**
Example 67 of the present application	6.60 (0.250)	7.02 ± 0.09 (0.095)	7.21 ± 0.07 (0.062)
Merck Example 3, enantiomer 1	5.72 (1.90)	6.06 ± 0.21 (0.87)	6.46 ± 0.05 (0.346)
Merck Example 3, enantiomer 2	7% inhibition @ 10 μM	25.4% inhibition @ 10 μM	49.6% activity @ 10 μM

The two constituent enantiomers derived from Merck Example 3 showed significantly different binding and functional activity at the D3 receptor, and unpredictably it was enantiomer 1 which showed the greater affinity. If screened as a racemic mixture (as disclosed in US 5077290) the apparent potency would be expected to be roughly 2x weaker in the binding assay than the binding activity for enantiomer 1 shown in the table. It is more difficult to predict accurately how the racemic mixture would perform in the functional assay, but based on the data for the individual enantiomers it would be expected to be weaker in activity than enantiomer 1.

The results shown in the table and in Figures 1 and 2 clearly show that Example 67 of the present application demonstrated notably higher affinity at the D3 receptor in binding (2 different assay formats) and higher potency in the functional assay, than the more active enantiomer (enantiomer 1). Therefore, even stronger activity for present Example 67 would be projected as compared to the racemic mixture of Merck Example 3.

Figure 1 - Results of D3 binding assay 2

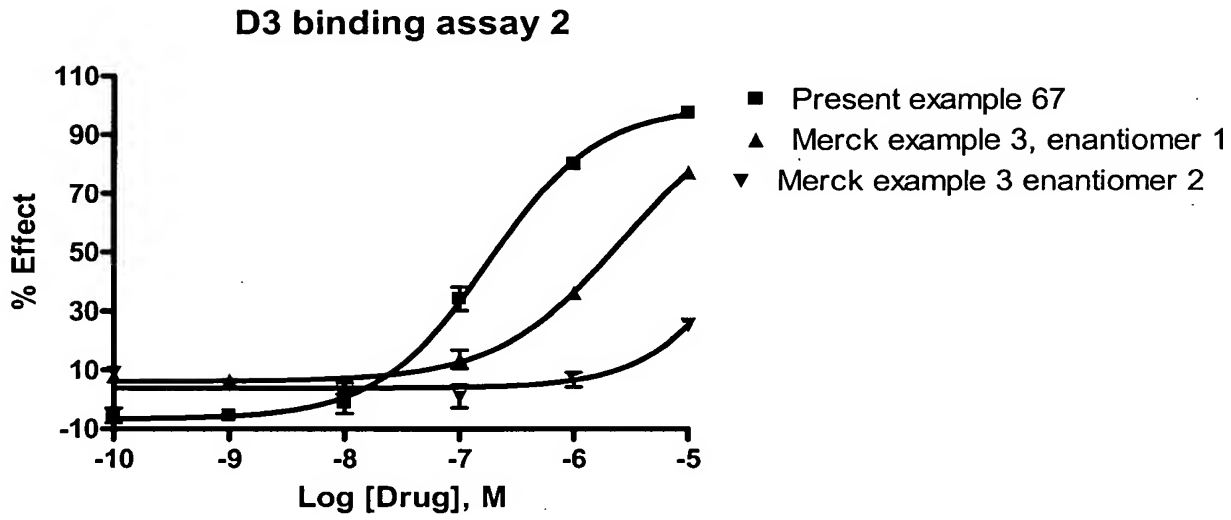
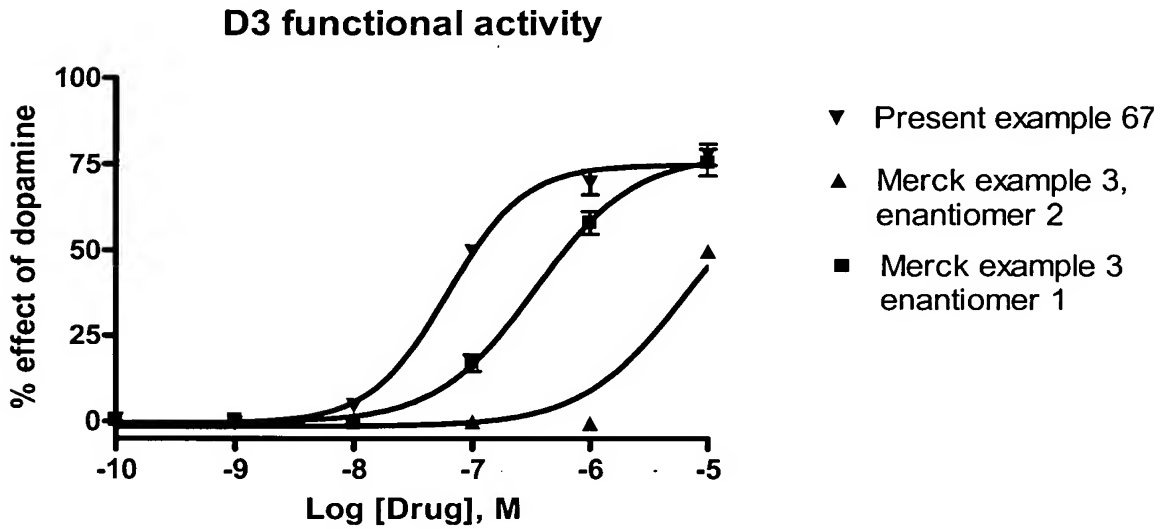


Figure 2 - Results of D3 functional assay



Appendix 1

GLP Study report for binding assay 1 for Example 67, completed at CEREP Biosciences

In this report, the compound of Example 67 of the present application is referred to by the reference number PF-592379.

Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

Cerep

STUDY NUMBER 884017
FINAL REPORT

BioPrint Profile

- Part A: PF-592379-00 Pfizer Reference Compound -

Annie OTTO-BRUC, Ph.D.

Paris
128 rue Danton
92500 Rueil-Malmaison
FRANCE
tel. +33 (0)1 55 94 84 00
fax +33 (0)1 55 94 84 10

Poitiers
Le Bois l'Evêque
86600 Celle l'Evescault
FRANCE
tel. +33 (0)5 49 89 30 00
fax +33 (0)5 49 43 21 70

Seattle
15318 NE 95th Street
Redmond, WA 98052
USA
tel. +1 (425) 895 8666
fax +1 (425) 895 8668

Tokyo
11th Floor Yurakucho Building
1-10-1 Yurakucho, Chiyoda-ku
Tokyo 100-006
JAPAN
tel. +81 (0)3 5219 2592
fax +81 (0)3 5219 2593

www.cerep.com
customer_support@cerep.fr

Cerep

Le Bois l'Evêque
B.P.1
86 600 Celle l'Evescault
France

www.cerep.com
Tel: 33 (0)5.49.89.30.00
Fax: 33 (0)5.49.43.21.70

Ref.: Final Report 884017/GG

STUDY NUMBER 884017

BioPrint Profile

- Part A: PF-592379-00 Pfizer Reference Compound -

Study Sponsor:	PFIZER GLOBAL RESEARCH & DEVELOPMENT
Attention:	Dr. Carolyn NAPIER *In Vitro* Team Leader
Address:	Candidate Research Group Building 500, Romm 1.579, IPC 388 SANDWICH Kent CT13 9NJ UNITED KINGDOM
Study Director:	Annie OTTO-BRUC, Ph.D.
Testing Facilities:	Cerep Le Bois l'Evêque - B.P. 1 - 86600 CELLE L'EVESCAULT, FRANCE and 15318 NE 95th Street, REDMOND, WA 98052 U.S.A.
Study Period:	From November 12, 2003 to February 24, 2004
Report Version:	1
Report Date:	August 12, 2004

STUDY DIRECTOR

Annie OTTO-BRUC, Ph.D.
Principal Scientist, Pharmacology

Date

CONTENTS

1. PURPOSE OF THE STUDY

The purpose of this study was to investigate the effects of PF-592379-00 in various *in vitro* receptor binding, cell biology and ADME-Tox assays.

2. MATERIALS AND METHODS

2.1. *IN VITRO* PHARMACOLOGY: Binding Assays

2.1.1. General Procedures

Assay	Origin	Reference Compound	Bibliography
	human recombinant (CHO cells)	DPCPX	Townsend-Nicholson and Schofield (1994)
	human recombinant (HEK-293 cells)	NECA	Luthin et al. (1995)
	human recombinant (HEK-293 cells)	IB-MECA	Salvatore et al. (1993)
	rat cerebral cortex	prazosin	Greengrass and Bremner (1979)
α_2 (non-selective)	rat cerebral cortex	yohimbine	Uhlen and Wikberg (1991)
	human recombinant (CHO cells)	yohimbine	Langin et al. (1989)
α_{2B}	NG 108-15 cells	yohimbine	Bylund et al. (1988)
	human recombinant (Sf9 cells)	atenolol	Smith and Teitler (1999)
	human recombinant (Sf9 cells)	ICI 118551	Smith and Teitler (1999)
	SK-N-MC cells	cyanopindolol	Curran and Fishman (1996)
	human recombinant (CHO cells)	saralasin	Bergsma et al. (1992)
	human recombinant (Hela cells)	saralasin	Tsuzuki et al. (1994)
BZD (central)	rat cerebral cortex	diazepam	Speth et al. (1979)
	human recombinant (CHO cells)	NPC 567	Pruneau et al. (1998)
	SK-N-MC cells	hCGRPα	Muff et al. (1992)
	human recombinant (HEK-293 cells)	WIN 55212-2	Matsuda et al. (1990)
	human recombinant (HEK-293 cells)	WIN 55212-2	Munro et al. (1993)
CCK_A *(h)* (CCK_1)	human recombinant (NIH-3T3 cells)	CCK-8	Talkad et al. (1994)

Assay	Origin	Reference Compound	Bibliography
CCK_B *(h)* (CCK_2)	human recombinant (HEK-293 cells)	CCK-8	Lee et al. (1993)
	human recombinant (L cells)	SCH 23390	Zhou et al. (1990)
	human recombinant (CHO cells)	(+)butaclamol	Grandy et al. (1989)
	human recombinant (CHO cells)	(+)butaclamol	Mackenzie et al. (1994)
	human recombinant (CHO cells)	clozapine	Van Tol et al. (1992)
	human recombinant (CHO cells)	endothelin-3	Buchan et al. (1994)
$GABA_A$	rat cerebral cortex	muscimol	Snodgrass (1978)
$GABA_B$	rat cerebral cortex	baclofen	Bowery et al. (1983)
	rat cerebral cortex	kainic acid	Monaghan and Cotman (1982)
	rat cerebral cortex	CGS 19755	Sills et al. (1991)
	rat cerebral cortex	glycine	Siegel et al. (1995)
	human recombinant (HEK-293 cells)	MIP-1α	Neote et al. (1993)
	human recombinant (HEK-293 cells)	ghrelin	Katugampola et al. (2001)
	guinea-pig cerebellum	pyrilamine	Dini et al. (1991)
H_2	guinea-pig striatum	cimetidine	Ruat et al. (1990)
H_3	rat cerebral cortex	(R)α-Me-histamine	Arrang et al. (1990)
I_1 (peripheral)	bovine adrenal medulla glands	rilmenidine	Dontenwill et al. (1999)
LTD_4 *(h)*	U-937 cells	LTD_4	Frey et al. (1993)
MC_1	B16-F1 cells	NDP-α-MSH	Siegrist et al. (1988)
	human recombinant (HEK-293 cells)	NDP-α-MSH	Schioth et al. (1997)
	chicken brain	melatonin	Rivkees et al. (1989)
ML_2 *(MT_3)*	hamster brain	melatonin	Pickering and Niles (1990)
	rat cerebral cortex	clorgyline	Cesura et al. (1990)
MAO-B	rat cerebral cortex	(R)-deprenyl	Cesura et al. (1989)

Assay	Origin	Reference Compound	Bibliography
	human recombinant (CHO cells)	pirenzepine	Dorje et al. (1991)
	human recombinant (CHO cells)	methoctramine	Dorje et al. (1991)
	human recombinant (CHO cells)	4-DAMP	Dorje et al. (1991)
	U-373MG cells	$[Sar^9,Met(O_2)^{11}]$-SP	Heuillet et al. (1993)
	human recombinant (Sf9 cells)	NPY	Munoz et al. (1995)
	rat cerebral cortex	nicotine	Pabreza et al. (1991)
N *(h)* (muscle-type)	TE671 cells	α-bungarotoxin	Lukas (1986)
δ_2 *(h)* (DOP)	human recombinant (CHO cells)	DPDPE	Simonin et al. (1994)
κ (KOP)	guinea-pig cerebellum	U 50488	Kinouchi and Pasternak (1991)
μ *(h)* (MOP)	human recombinant (CHO cells)	DAMGO	Wang et al. (1994)
ORL1 *(h)* (NOP)	human recombinant (HEK-293 cells)	nociceptin	Ardati et al. 1997)
OT *(h)*	ECV-304 cells	oxytocin	Thibonnier et al. (1999)
PCP	rat cerebral cortex	MK 801	Vignon et al. (1986)
	rat urinary bladder	α,β-MeATP	Bo and Burnstock (1990)
	human recombinant (HEK-293 cells)	8-OH-DPAT	Mulheron et al. (1994)
5-HT_{1B}	rat cerebral cortex	5-HT	Hoyer et al. (1985)
	bovine caudate	serotonin	Heuring and Peroutka (1987)
	human recombinant (HEK-293 cells)	ketanserin	Bonhaus et al. (1995)
	human recombinant (CHO cells)	serotonin	Bonhaus et al. (1995)
	human recombinant (CHO cells)	SB 242084	Stam et al. (1994)
	human recombinant (HEK-293 cells)	MDL 72222	Hope et al. (1996)
	human recombinant (CHO cells)	5-HT	Mialet et al. (2000)

Assay	Origin	Reference Compound	Bibliography
	human recombinant (HEK-293 cells)	serotonin	Monsma et al. (1993)
	human recombinant (CHO cells)	serotonin	Shen et al. (1993)
σ (non-selective)	rat cerebral cortex	haloperidol	Shirayama et al. (1993)
	human recombinant (HEK-293 cells)	somatostatin	Rohrer et al. (1993)
	human recombinant (HEK-293 cells)	somatostatin	Yamada et al. (1993)
	IM-9 cells (cytosol)	dexamethasone	Clark et al. (1996)
Estrogen α *(h)* (ERα)	human recombinant (Sf9 cells)	17-β-estradiol	Parker et al. (2000)
Androgen *(h)* (AR)	LNCaP cells (cytosol)	methyltrienolone	Zava et al. (1979)
	rat liver	T_3	Inoue et al. (1983)
Urotensin-II (UT-II)	mouse recombinant (CHO cells)	urotensin-II	Liu et al. (1999)
VIP_1 *(h)* ($VPAC_1$)	human recombinant (CHO cells)	VIP	Couvineau et al. (1985)
	human recombinant (CHO cells)	$[d(CH_2)_5{}^1,Tyr(Me)_2]$-AVP	Tahara et al. (1998)
Ca^{2+} channel (L, DHP site)	rat cerebral cortex	nitrendipine	Lee et al. (1984)
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	rat cerebral cortex	D 600	Reynolds et al. (1986)
Ryanodine (RY_3)	rat cerebral cortex	ryanodine	Padua et al. (1992)
K^+_{ATP} channel	rat cerebral cortex	glibenclamide	Angel and Bidet 1991)
	rat cerebral cortex	α–dendrotoxin	Sorensen and Blaustein (1989)
SK^+_{Ca} channel	rat cerebral cortex	apamin	Hugues et al. (1982)
Na^+ channel (site 2)	rat cerebral cortex	veratridine	Brown (1986)
Cl^- channel	rat cerebral cortex	picrotoxinin	Lewin et al. (1989)
	human recombinant (MDCK cells)	protriptyline	Pacholczyk et al. (1991)

Assay	Origin	Reference Compound	Bibliography
	human recombinant (CHO cells)	BTCP	Andersen (1987)
GABA transporter	rat cerebral cortex	nipecotic acid	Shank et al. (1990)
	rat striatum	hemicholinium-3	Vickroy et al. (1984)
	human recombinant (HEK-293 cells)	imipramine	Tatsumi et al. (1997)

2.1.2. Experimental Conditions

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
	[^{3}H]DPCPX	1 nM	DPCPX (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]CGS 21680	6 nM	NECA (10 µM)	90 min./22°C	Scintillation counting
	[^{125}I]AB-MECA	0.1 nM	IB-MECA (1 µM)	90 min./22°C	Scintillation counting
	[^{3}H]prazosin	0.25 nM	prazosin (0.5 µM)	60 min./22°C	Scintillation counting
	[^{3}H]RX 821002	0.5 nM	(-)epinephrine (100 µM)	30 min./22°C	Scintillation counting
	[^{3}H]RX 821002	1 nM	(-)epinephrine (100 µM)	30 min./22°C	Scintillation counting
	[^{3}H]RX 821002	2.5 nM	(-)epinephrine (100 µM)	25 min./22°C	Scintillation counting
	[^{3}H](-)CGP 12177	0.15 nM	alprenolol (50 µM)	60 min./22°C	Scintillation counting
	[^{3}H](-)CGP 12177	0.15 nM	alprenolol (50 µM)	60 min./22°C	Scintillation counting
	[^{125}I]CYP (+ 1 µM (-)propranolol)	0.6 nM	(-)propranolol (1 mM)	90 min./37°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
	[^{125}I][Sar^1,Ile^8]-AT II	0.05 nM	angiotensin II (10 µM)	60 min./37°C	Scintillation counting
	[^{125}I]CGP 42112A	0.05 nM	angiotensin II (1 µM)	180 min./37°C	Scintillation counting
	[^{3}H]flunitrazepam	0.4 nM	diazepam (3 µM)	60 min./4°C	Scintillation counting
	[^{3}H]bradykinin	0.2 nM	bradykinin (1 µM)	90 min./22°C	Scintillation counting
	[^{125}I]hCGRPα	0.04 nM	hCGRPα (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]WIN 55212-2	2 nM	WIN 55212-2 (10 µM)	90 min./37°C	Scintillation counting
	[^{3}H]WIN 55212-2	0.8 nM	WIN 55212-2 (5 µM)	90 min./30°C	Scintillation counting
	[^{125}I]CCK-8	0.08 nM	CCK-8 (1 µM)	60 min./22°C	Scintillation counting
	[^{125}I]CCK-8	0.025 nM	CCK-8 (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]SCH 23390	0.3 nM	SCH 23390 (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C	Scintillation counting
	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C	Scintillation counting
	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C	Scintillation counting
	[^{125}I]endothelin-1	0.03 nM	endothelin-1 (0.1 µM)	120 min./37°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
	[^{3}H]muscimol	5 nM	muscimol (10 µM)	10 min./4°C	Scintillation counting
	[^{3}H]GABA (+ 40 µM isoguvacine)	10 nM	baclofen (100 µM)	10 min./22°C	Scintillation counting
	[^{3}H]kainic acid	5 nM	L-glutamate (1 mM)	60 min./4°C	Scintillation counting
	[^{3}H]CGP 39653	5 nM	L-glutamate (100 µM)	60 min./4°C	Scintillation counting
Glycine (strychnine-insensitive)	[^{3}H]MDL 105,519	0.5 nM	glycine (1 mM)	45 min./0°C	Scintillation counting
	[^{125}I]MIP-1α	0.03 nM	MIP-1α (0.1 µM)	90 min./22°C	Scintillation counting
	[^{125}I][His]-ghrelin	0.02 nM	ghrelin (0.1 µM)	30 min./22°C	Scintillation counting
	[^{3}H]pyrilamine	0.5 nM	triprolidine (100 µM)	10 min./22°C	Scintillation counting
	[^{125}I]APT	0.1 nM	tiotidine (100 µM)	150 min./22°C	Scintillation counting
	[^{3}H](R)α-Me-histamine	1 nM	(R)α-Me-histamine (5 µM)	120 min./22°C	Scintillation counting
	[^{3}H]clonidine (+ 10 µM RX821002)	15 nM	rilmenidine (10 µM)	30 min./22°C	Scintillation counting
	[^{3}H]LTD$_4$	0.3 nM	LTD$_4$ (1 µM)	60 min./22°C	Scintillation counting
	[^{125}I]NDP-α-MSH	0.05 nM	NDP-α-MSH (1 µM)	90 min./22°C	Scintillation counting
	[^{125}I]NDP-α-MSH	0.05 nM	NDP-α-MSH (1 µM)	60 min./37°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
	[^{125}I]iodomelatonin	0.025 nM	melatonin (1 µM)	60 min./22°C	Scintillation counting
	[^{125}I]iodomelatonin	0.1 nM	melatonin (30 µM)	30 min./4°C	Scintillation counting
	[^{3}H]Ro 41-1049	10 nM	clorgyline (1 µM)	60 min/37°C	Scintillation counting
	[^{3}H]Ro 19-6327	15 nM	(R)-deprenyl (10 µM)	90 min./22°C	Scintillation counting
	[^{3}H]pirenzepine	2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]AF-DX 384	2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]4-DAMP	0.2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting
	[^{125}I][Sar^{9},Met(O$_2$)^{11}]-SP	0.15 nM	[Sar^{9},Met(O$_2$)^{11}]-SP (1 µM)	60 min./22°C	Scintillation counting
	[^{125}I]peptide YY	0.05 nM	NPY (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]cytisine	1.5 nM	nicotine (10 µM)	75 min./4°C	Scintillation counting
	[^{125}I]α-bungarotoxin	2.5 nM	α-bungarotoxin (5 µM)	120 min./22°C	Scintillation counting
	[^{3}H]DADLE	0.5 nM	naltrexone (10 µM)	120 min./22°C	Scintillation counting
	[^{3}H]U 69593	0.7 nM	naloxone (10 µM)	80 min./22°C	Scintillation counting
	[^{3}H]DAMGO	0.5 nM	naloxone (10 µM)	150 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
	[^{3}H]nociceptin	0.2 nM	nociceptin (1 µM)	60 min./22°C	Scintillation counting
	[^{125}I]OVTA	0.3 nM	oxytocin (10 µM)	30 min./30°C	Scintillation counting
	[^{3}H]TCP	5 nM	MK 801 (10 µM)	45 min./22°C	Scintillation counting
	[^{3}H]α,β-MeATP	3 nM	α,β-MeATP (10 µM)	120 min./4°C	Scintillation counting
	[^{3}H]8-OH-DPAT	0.5 nM	8-OH-DPAT (10 µM)	60 min./22°C	Scintillation counting
	[^{125}I]CYP	0.1 nM	serotonin (10 µM)	90 min./37°C	Scintillation counting
	[^{3}H]serotonin	2 nM	serotonin (10 µM)	30 min./22°C	Scintillation counting
	[^{3}H]ketanserin	0.5 nM	ketanserin (1 µM)	15 min./37°C	Scintillation counting
	[^{3}H]LSD	1.2 nM	serotonin (10 µM)	30 min/37°C	Scintillation counting
	[^{3}H]mesulergine	1 nM	SB 242084 (10 µM)	30 min./37°C	Scintillation counting
	[^{3}H]BRL 43694	0.5 nM	MDL 72222 (10 µM)	60 min./22°C	Scintillation counting
	[^{3}H]GR 113808	0.2 nM	serotonin (100 µM)	30 min./37°C	Scintillation counting
	[^{3}H]LSD	2 nM	serotonin (100 µM)	60 min./37°C	Scintillation counting
	[^{3}H]LSD	4 nM	serotonin (10 µM)	120 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
	[^{3}H]DTG	8 nM	haloperidol (10 µM)	120 min./22°C	Scintillation counting
	[^{125}I]Tyr^{11}-somatostatin	0.2 nM	somatostatin (1 µM)	60 min./22°C	Scintillation counting
	[^{125}I]Tyr^{11}-somatostatin	0.05 nM	somatostatin (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H]triamcinolone	1.5 nM	dexamethasone (10 µM)	18 h./4°C	Scintillation counting
	fluormone^{TM}ES2	1 nM	17-β-estradiol (1 µM)	120 min./22°C	Fluorescence polarization
	[^{3}H]methyltrienolone	0.5 nM	mibolerone (1 µM)	24 h./4°C	Scintillation counting
	[^{125}I]T_3	0.1 nM	T_3 (1 µM)	18 h./4°C	Scintillation counting
	[^{125}I]urotensin-II	0.1 nM	urotensin-II (3 µM)	60 min./22°C	Scintillation counting
	[^{125}I]VIP	0.04 nM	VIP (0.3 µM)	60 min./22°C	Scintillation counting
	[^{3}H]AVP	0.3 nM	AVP (1 µM)	60 min./22°C	Scintillation counting
	[^{3}H](+)PN 200-110	0.04 nM	nifedipine (1 µM)	90 min./22°C	Scintillation counting
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	[^{3}H](-)D 888	0.5 nM	D 600 (10 µM)	60 min./22°C	Scintillation counting
	[^{3}H]ryanodine	3 nM	ryanodine (10 µM)	120 min./37°C	Scintillation counting
	[^{3}H]glibenclamide	0.1 nM	glibenclamide (1 µM)	60 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
	[^{125}I]α–dendrotoxin	0.01 nM	α–dendrotoxin (50 nM)	30 min./22°C	Scintillation counting
	[^{125}I]apamin	0.004 nM	apamin (0.1 μM)	30 min./0°C	Scintillation counting
	[^{3}H]batrachotoxinin	10 nM	veratridine (300 μM)	60 min./22°C	Scintillation counting
	[^{35}S]TBPS	3 nM	picrotoxinin (20 μM)	90 min./22°C	Scintillation counting
	[^{3}H]nisoxetine	1 nM	desipramine (1 μM)	60 min/4°C	Scintillation counting
	[^{3}H]GBR12935	0.5 nM	BTCP (10 μM)	120 min./4°C	Scintillation counting
	[^{3}H]GABA (+ 10 μM isoguvacine) (+ 10 μM baclofen)	10 nM	GABA (1 mM)	30 min./22°C	Scintillation counting
	[^{3}H]hemicholinium-3	3 nM	hemicholinium-3 (10 μM)	30 min./22°C	Scintillation counting
	[^{3}H]paroxetine	0.1 nM	imipramine (10 μM)	30 min./22°C	Scintillation counting

2.1.3. Analysis and Expression of Results

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand.

The results are expressed as a percent of control specific binding and as a percent inhibition of control specific binding obtained in the presence of PF-592379-00.
Individual and mean values are presented in the results section.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (n_H) were determined by non-linear regression analysis of the competition curves using Hill equation curve fitting.

The inhibition constants (K_i) were calculated from the Cheng Prusoff equation ($K_i = IC_{50}/(1+(L/K_D))$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor).

2.2. *IN VITRO* PHARMACOLOGY: Enzyme and Cell-based Assays

2.2.1. General Procedures

Assay	Origin	Reference Compound	Bibliography
	HUV-EC-C cells	NS 398	Miralpeix et al. (1997)
	RAW 264-7 cells	1400W	Tayeh and Marletta (1989)
Phosphodiesterase 2 *(h)*	differentiated U-937 cells	EHNA	Torphy et al. (1992)
Phosphodiesterase 3 *(h)*	human platelets	milrinone	Weishaar et al. (1986)
	U-937 cells	rolipram	Torphy et al. (1992)
Phosphodiesterase 5 *(h)*	human platelets	dipyridamole	Weishaar et al. (1986)
	bovine retina	zaprinast	Ballard et al. (1998)
Phosphodiesterase 11 (h)-Pfizer	Pfizer	dipyridamole	Fawcett et al. (2000)
ACE *(h)* (recombinant)	human recombinant (murine cells)	captopril	Hoorn and Roth (1993)
Elastase *(h)*	human leukocytes	3',4'dichloroisocoumarin	Adeyemi et al. (1990)
	human recombinant (*E. coli*)	pepstatin A	Toth and Marshall (1990)
Neutral endopeptidase *(h)*	HUV-EC-C cells	thiorphan	Graf et al. (1998)
	human recombinant (*E. coli*)	GM6001	Bickett et al. (1993)
MMP-2 *(h)*	human recombinant	GM6001	Nagase et al. (1994)
	human recombinant (Sf9 cells)	GM6001	Nagase et al. (1994)
	human recombinant (*E. coli*)	GM6001	Quesada et al. (1997)
MMP-9 *(h)*	human recombinant	GM6001	Nagase et al. (1994)
	human lung	leupeptin	Schwartz and Bradford (1986)
Guanylyl cyclase (basal)	bovine lung	sodium nitroprusside	Wolin et al. (1982)
	human recombinant (*E. coli*)	Na_3VO_4	Chevalier et al. 1988)
	mouse recombinant (*E.coli*)	staurosporine	Parker et al. (2000)
CAM kinase II	rat brain	staurosporine	Lengyel et al. (2001)
	rat recombinant (*E. coli*)	staurosporine	Robbins et al. (1993)

Assay	Origin	Reference Compound	Bibliography
$p56^{lyn}$ kinase	bovine spleen	staurosporine	Parker et al. (2000)
$p55^{fyn}$ kinase	bovine thymus	staurosporine	Cheng et al. (1992)
	human recombinant (insect cells)	staurosporine	Parker et al. (2000)
D4.4 receptor - G protein coupling *(h)* (agonist effect)	human recombinant (CHO cells)	dopamine	Chio et al. (1994)
D4.4 receptor - G protein coupling *(h)* (antagonist effect)	human recombinant (CHO cells)	spiperone	Chio et al. (1994)
	human recombinant (HEK-293 cells)	neostigmine	Ellman et al. (1961)
Catechol-O-methyl transferase	porcine liver	Ro 41-0960	Muller-Enoch et al. (1976)
	rat brain	AoAA	Losher (1981)
ATPase (Na^+/K^+)	dog kidney	ouabain	Fiske and Subbarow (1925)

2.2.2. Experimental Conditions

Assay	Substrate/Stimulus/Tracer	Incubation	Reaction Product	Method of Detection
COX_2 *(h)* (isolated enzyme)	arachidonic acid (1 µM)	10 min./25°C	PGE_2	EIA
inducible NOS (isol. enz/ spectrophoto.)	arginine (100 µM)	3 h./37°C	NO_2^-	Photometry
	[^{3}H]cAMP + cAMP (1 µM)	30 min./30°C	[^{3}H]5'AMP	Scintillation counting
	[^{3}H]cAMP + cAMP (0.1 µM)	30 min./30°C	[^{3}H]5'AMP	Scintillation counting
	[^{3}H]cAMP + cAMP (1 µM)	30 min./30°C	[^{3}H]5'AMP	Scintillation counting
	[^{3}H]cGMP + cGMP (1 µM)	30 min./30°C	[^{3}H]5'GMP	Scintillation counting
	[^{3}H]cGMP + cGMP (2 µM)	30 min./30°C	[^{3}H]5'GMP	Scintillation counting

Assay	Substrate/Stimulus/Tracer	Incubation	Reaction Product	Method of Detection
	[^{3}H]GMPc + GMPc (10 µM)	60 min./30°C	[^{3}H]5'GMP	Scintillation counting
	Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys (DNP)-OH (10 µM)	20 min./22°C	Mca-peptides	Fluorimetry
	MeOSAAPV-pNa (0.1 mM)	60 min./37°C	pNa	Photometry
	antranilyl-HIV (75 µM)	40 min./37°C	N-terminal tripeptide	Fluorimetry
	DAGNPG (50 µM)	60 min./37°C	Dansyl-D-Ala-Gly	Fluorimetry
	DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(n-Me-Abz)-NH_2 (10 µM)	40 min./37°C	Cys(Me)-His-Ala-Lys(n-Me-Abz)-NH_2	Fluorimetry
	NFF-2 (10 µM)	90 min./37°C	Mca-Arg-Pro-Lys-Pro-Tyr-Ala	Fluorimetry
	NFF-2 (10 µM)	60 min./RT	Mca-Arg-Pro-Lys-Pro-Tyr-Ala	Fluorimetry
	MMP-2/MMP-7 substrate (5 µM)	45 min./37°C	Mca-Pro-Leu-Gly	Fluorimetry
	NFF-2 (5 µM)	45 min./22°C	Mca-Arg-Pro-Lys-Pro-Tyr-Ala	Fluorimetry
	N-p-Tosyl-Gly-Pro-Arg-p-nitroanilide (0.1 mM)	8 min/37°C	p-nitroanilide	Photometry
Guanylyl cyclase (basal)	GTP (0.1 mM)	15 min./30°C	cGMP	RIA
	pNPP (0.6 mM)	30 min./22°C	pNP	Photometry
	poly GT (0.4 µg/ml)	15 min./30°C	phosphopoly GT	Fluorescence polarization
	[γ-^{33}P]ATP + autocamtide-2 (5 µM)	40 min./22°C	[γ^{33}P]autocamtide-2	Scintillation counting
	[γ^{33}P]ATP + MBP (0.5 mg/ml)	30 min./37°C	[γ^{33}P]MBP	Scintillation counting

Assay	Substrate/Stimulus/Tracer	Incubation	Reaction Product	Method of Detection
	KVEKIGEGTYGVVYK (300 µM)	45 min./30°C	phosphoKVEKIGEGTYGVVYK	Fluorescence polarization
	[γ-^{33}P]ATP + poly GT (0.5 mg/ml)	30 min/25°C	[γ-^{33}P]poly GT	Scintillation counting
	poly GT (0.3 µg/ml)	15 min./22°C	phosphopoly GT	Fluorescence polarization
D4.4 receptor - G protein coupling *(h)* (agonist effect)	none (10 µM dopamine for control)	30 min./30°C	[^{35}S]GTP-γ-S binding	Scintillation counting
D4.4 receptor - G protein coupling *(h)* (antagonist effect)	dopamine (0.3 µM)	30 min./30°C	[^{35}S]GTP-γ-S binding	Scintillation counting
	AMTCh (50 µM)	30 min./37°C	thio-conjugate	Photometry
Catechol-O-methyl transferase	esculetin (1 µM)	30 min./37 °C	scopoletin	Fluorimetry
	GABA (9 mM) + α-ketoglutarate (9 mM)	60 min./37°C	succinic semialdehyde	Fluorimetry
	ATP (2 mM)	60 min./37°C	Pi	Photometry

2.2.3. Analysis and Expression of Results

The results are expressed as a percent of control values and as a percent variation of control values obtained in the presence of PF-592379-00.
Individual and mean values are presented in the results section.

The IC_{50} values (concentration causing a half-maximal inhibition of control values), EC_{50} values (concentration causing a half-maximal stimulation of control values) and Hill coefficients (n_H) were determined by non-linear regression analysis of the concentration-response curves using Hill equation curve fitting.

2.3. ADME-Tox: Solution Properties

2.3.1. General Procedures

Assay	Technique	Additional Information	Reference Compound	Bibliography
	Shake-Flask	Chromatographic purity UV/VIS spectrum	8 Reference compounds	Lipinski et al. (1997)
Partition Coefficient *(log D, n-octanol/PBS, pH 7.4)*	Shake-Flask		8 Reference compounds	Sangster (1997)
Partition Coefficient *(log D, cyclohexane/PBS, pH 7.4)*	Shake-Flask		8 Reference compounds	Young et al. (1998)

Notes:
8 Reference compounds: metoprolol, rifampicin, ketoconazole, phenytoin, haloperidol, simvastatin, diethylstilbestrol, and tamoxifen.

2.3.2. Experimental Conditions

Assay	Test Compound	Equilibration / Incubation	Analytical Method
	200 µM (n=2) 2 % DMSO	24 hours in PBS at pH 7.4 at RT	HPLC-UV/VIS
Partition Coefficient *(log D, n-octanol/PBS, pH 7.4)*	100 µM (n=3) 1 % DMSO	60 min in n-octanol-PBS at pH 7.4 at RT	HPLC-UV/VIS
	100 µM (n=3) 1 % DMSO	60 min in n-cyclohexane-PBS at pH 7.4 at RT	HPLC-UV/VIS

Notes:
For the solubility assay, the default detection wavelength (230 nm) may be substituted, if appropriate.
For the partition coefficient assay, the optimized detection wavelength is based on the UV/VIS spectrum acquired during the aqueous solubility assay.

Abbreviations:
DMSO: Dimethylsulfoxide
HPLC-UV/VIS: HPLC with photodiode array detection (Instrumentation: Dionex)
HPLC: High performance liquid chromatography
PBS: Phosphate buffered saline; from Sigma, catalog number D-5652
RT: Room temperature
UV/VIS: Ultraviolet/Visible

2.3.3. Analysis and Expression of Results

Aqueous Solubility

Aqueous solubility (µM) was determined by comparing the peak area of the principal peak in a calibration standard (200 µM) containing organic solvent (methanol/water, 60/40, v/v) with the peak area of the corresponding peak in a buffer sample. In addition, chromatographic purity (%) was defined as the peak area of the principal peak relative to the total integrated peak area in the HPLC chromatogram of the calibration standard. A chromatogram of the calibration standard of the test compound, along with a UV/VIS spectrum with labeled absorbance maxima, was generated.

Partition Coefficient (Log D, pH 7.4)

The total amount of compound was determined as the peak area of the principal peak in a calibration standard (100 µM) containing organic solvent (methanol/water, 60/40, v/v). The amount of compound in buffer was determined as the combined, volume-corrected, and weighted areas of the corresponding peaks in the buffer phases of three octanol-buffer samples of different composition. An automated weighting system was used to ensure the preferred use of raw data from those samples with well quantifiable peak signals. The amount of compound in octanol was calculated by subtraction. Subsequently, Log D was calculated as the Log_{10} of the amount of compound in the octanol phase divided by the amount of compound in the buffer phase.

2.4. ADME-Tox: Bioanalytical

2.4.1. General Procedures

Assay	Technique	Additional Information
HPLC-MS Screen	HPLC-MS, and HPLC-MS/MS	Full scan and product ion spectra; SRM conditions for quantitation, and ionization potential

2.4.2. Experimental Conditions

Assay	Test Compound	Analytical method
	200 µM (n = 1) acetonitrile/methanol/water (25/25/50, v/v/v)	HPLC-MS and HPLC-MS/MS

Abbreviations:
HPLC-MS/MS: HPLC coupled with tandem mass spectrometry (Instrumentation: Thermo Finnigan)
HPLC-MS: HPLC with mass spectrometry detection (Instrumentation: Thermo Finnigan)
HPLC: High performance liquid chromatography
SRM: Selected reaction monitoring

2.4.3. Analysis and Expression of Results

HPLC-MS Screen

Full scan HPLC-MS analysis was conducted on the test compound at 200 µM. Total ion current chromatograms and corresponding mass spectra were generated for the test compound in both positive and negative ionization modes. The precursor ions for MS/MS were selected from either the positive or the negative mass spectrum, as a function of the respective ion abundance. In addition, product ion HPLC-MS/MS analysis was performed in order to determine the appropriate selected fragmentation reaction for use in quantitative analysis. The final reaction monitoring parameters were chosen to maximize the possibility for quantitation of the test compound when present within a complex mixture of components. Finally, the test compound was assigned a rank number of ionization, which directly indicates its ease of quantitation.

2.5. ADME-Tox: *In Vitro* Absorption

2.5.1. General Procedures

Assay	Cell	Passage Number	Days in Culture	Reference Compound	Bibliography
A-B Permeability *(pH 6.5/7.4)*	TC7	15 passages in culture between passages 20 and 40	13 to 25	propranolol, ranitidine, vinblastine*	Gres et al. (1998)
A-B Permeability *(pH 7.4/7.4)*	TC7	15 passages in culture between passages 20 and 40	13 to 25	propranolol, ranitidine, vinblastine*	Gres et al. (1998)
B-A Permeability *(pH 6.5/7.4)*	TC7	15 passages in culture between passages 20 and 40	13 to 25	propranolol, ranitidine, vinblastine*	Hunter et al. 1993
B-A Permeability *(pH 7.4/7.4)*	TC7	15 passages in culture between passages 20 and 40	13 to 25	propranolol, ranitidine, vinblastine*	Hunter et al. 1993
	TC7	15 passages in culture between passages 20 and 40	13 to 25	verapamil	Cavet et al. (1996)

Notes:
TC7 is a sub-clone of the Caco-2 cell line.
* Vinblastine is tested in the A-B permeability when the B-A permeability is also requested.

2.5.2. Experimental Conditions

Assay	Test Concentration	Biological Conditions	Analytical Method
	50 µM in HBSS (n=2) 1 % DMSO	A-to-B flux at 37 °C with shaking 24-well transwell plate pH 6.5 in A and pH 7.4 in B Donor samples: time 0 and 120 min Receiver samples: time 60 min	HPLC-MS/MS
	50 µM in HBSS (n=2) 1 % DMSO	A-to-B flux at 37 °C with shaking 24-well transwell plate pH 7.4 in A and pH 7.4 in B Donor samples: time 0 and 120 min Receiver samples: time 120 min	HPLC-MS/MS
	50 µM in HBSS (n=2) 1 % DMSO	B-to-A flux at 37 °C with shaking 24-well transwell plate pH 6.5 in A and pH 7.4 in B Donor samples: time 0 and 60 min Receiver samples: time 60min	HPLC-MS/MS

Assay	Test Concentration	Biological Conditions	Analytical Method
	50 µM in HBSS (n=2) 1 % DMSO	B-to-A flux at 37 °C with shaking 24-well transwell plate pH 7.4 in A and pH 7.4 in B Donor samples: time 0 and 60min Receiver samples: time 60 min	HPLC-MS/MS
	50 µM in HBSS (n=3) 1 % DMSO in A and B sides	B-to-A flux at 37 °C with shaking 96-well transwell plate pH 7.4 in A and B sides Donor samples: time 0 and 180 min Receiver samples: time 180 min	Scintillation counting

Notes:
Transwell plate: from Costar, 24-well plate, catalog number 3399
Multiscreen plate: from Millipore, 96-well plate, catalog number MACAC02S5.

Abbreviations:
A: Apical side
B: Basolateral side
DMSO: Dimethylsulfoxide
HBSS: Hank's balanced salt solution, from Invitrogen, catalog number 11201
HEPES: N-(2-hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid)
HPLC-MS/MS: HPLC coupled with tandem mass spectrometry (Instrumentation: Thermo Finnigan)
HPLC-UV/VIS: HPLC with photodiode array detection (Instrumentation: Dionex)
HPLC: High performance liquid chromatography
MES: 2-(N-Morpholino)-ethanesulfonic acid, from Sigma, catalog number M-8652

A-B Permeability:

The working solution for the test compound was prepared as specified in the above table.
^{14}C-mannitol (approximately 4 µM) was also included in the working solution. The working solution was then added to the apical side. The respective buffer was added to the basolateral side.
The following parameter was used for assessing the cell monolayer integrity:
^{14}C-D-Mannitol permeability $< 2.5\times10^{-6}$ cm/s

B-A Permeability:

The working solution for the test compound was prepared as specified in the above table.
The working solution was then added to the basolateral side. The respective buffer, containing ^{14}C-D-mannitol (approximately 4 µM), was added to the apical side.
The following parameter was used for assessing the cell monolayer integrity:
^{14}C-D-Mannitol permeability $< 2.5\times10^{-6}$ cm/s

P-glycoprotein Inhibition:

Working solutions I and II were prepared for each test compound as follows:

Working Solution I: The compound was prepared at 50 µM in HBSS-HEPES (5 mM), at pH 7.4 from a 10 mM DMSO stock solution. Digoxin (10 µM) and 0.1 % BSA were included in this working solution. The working solution was then added to the apical side. Working Solution II: The compound was prepared at 50 µM in HBSS-HEPES (5 mM), at pH 7.4 from a 10 mM DMSO stock solution. ^{3}H-digoxin (10 µM) and 0.1 % BSA were included in this working solution. The working solution was then added to the basolateral side.

The following parameters were used for assessing the cell monolayer integrity:

Lucifer yellow (100 µM applied to the apical side) Papp < 1 x 10^{-6} cm/sec.

2.5.3. Analysis and Expression of Results

A-B Permeability

The apparent permeability coefficient (P_{app}) of the test compound in the apical to the basolateral direction was calculated as follows.

$$P_{app}(cm/s) = \frac{V_R \times C_{R120}}{\Delta t} \times \frac{1}{A \times (C_{D,mid} - C_{R,mid})}$$

where V_R is the volume of the receiver chamber. C_{R120} is the concentration of the test compound in the receiver chamber at time 120 minutes, Δt is the incubation time (120 minutes) and A is the surface area of the TC7 cell monolayer. $C_{D,mid}$ is the calculated mid-point concentration of the test compound in the donor side, which is the mean value of the donor concentration at time 0 minute and the donor concentration at time 120 minutes. $C_{R,mid}$ is the mid-point concentration of the test compound in the receiver side, which is one half of the receiver concentration at time 120 minutes. Concentrations of the test compound are expressed as peak areas of the test compound.

Recovery of the Test Compound from A-B Permeability Assay

The recovery of the test compound was calculated as follows:

$$\text{Recovery (\%)} = \frac{V_D \times C_{D120} + V_R \times C_{R120}}{V_D \times C_{WS}} \times 100$$

where V_D and V_R are the volumes of the donor and receiver chambers, respectively. C_{D120} is the concentration of the test compound in the donor sample at time 120 minutes. C_{R120} is the concentration of the test compound in the receiver sample at time 120 minutes. C_{WS} is the concentration of the test compound in the working solution. Concentrations of the test compound are expressed as peak areas of the test compound.

B-A Permeability

The apparent permeability coefficient (P_{app}) of the test compound in the basolateral to the apical direction was calculated as follows.

$$P_{app}\,(cm/s) = \frac{V_R \times C_{R60}}{\Delta t} \times \frac{1}{A \times (C_{D,mid} - C_{R,mid})}$$

where V_R is the volume of the receiver chamber. C_{R60} is the concentration of the test compound in the receiver chamber at time 60 minutes. Δt is the incubation time (60 minutes). A is the surface area of the TC7 cell monolayer. $C_{D,mid}$ is the calculated mid-point concentration of the test compound in the donor side, which is the mean value of the donor concentration at time 0 minute and the donor concentration at time 60 minutes. $C_{R,mid}$ is the mid-point concentration of the test compound in the receiver side, which is one half of the receiver concentration at time 60 minutes. Concentrations of the test compound are expressed as peak areas of the test compound.

Recovery of the Test Compound from B-A Permeability Assay

The recovery of the test compound was calculated as follows:

$$\text{Recovery (\%)} = \frac{V_D \times C_{D60} + V_R \times C_{R60}}{V_D \times C_{WS}} \times 100$$

where V_D and V_R are the volumes of the donor and receiver chambers, respectively. C_{D60} is the concentration of the test compound in the donor sample at time 60 minutes. C_{R60} is the concentration of the test compound in the receiver sample at time 60 minutes. C_{WS} is the concentration of the test compound in the working solution. Concentrations of the test compound are expressed as peak areas of the test compound.

P-glycoprotein Inhibition

The percent inhibition of the permeation of ^{3}H-digoxin was calculated as follows:

$$\text{Inhibition (\%)} = 100 - \left(\frac{(Value)_{test} - (Mean)_{background}}{(Mean)_{control} - (Mean)_{background}} \times 100\right)$$

where $(Mean)_{control}$ is the mean scintillation count of ^{3}H-digoxin on the apical side, obtained in the absence of the test compound. $(Value)_{test}$ is an individual scintillation count of ^{3}H-digoxin on the apical side, obtained in the presence of the test compound. $(Mean)_{background}$ is the mean scintillation count of ^{3}H-digoxin on the apical side, obtained in the presence of the highest concentration of the reference compound. This represents the value for passive permeation of ^{3}H-digoxin that cannot be inhibited by verapamil at 200 µM. The average % Pgp inhibition of two individual replicates is then reported.

2.6. ADME-Tox: *In Vitro* Metabolism

2.6.1. General Procedures

Assay	Source	Reference Compound	Bibliography
	Human recombinant (1.25 pmol/mL)	furafylline	Crespi et al. (1997)
CYP2B6 Inhibition *(EFC substrate)*	Human recombinant (10 pmol/mL)	ketoconazole	Ekins et al. (1997)
CYP2C9 Inhibition *(7-MFC substrate)*	Human recombinant (15 pmol/mL)	sulfaphenazole	Crespi et al. (1997)
CYP2C19 Inhibition *(CEC substrate)*	Human recombinant (10 pmol/mL)	tranylcypromine	Ono et al. (1996)
CYP2D6 Inhibition *(AMMC substrate)*	Human recombinant (15 pmol/mL)	quinidine	Ono et al. (1996)
CYP2E1 Inhibition *(7-EC substrate)*	Human recombinant (15 pmol/mL)	4-methylpyrazole	Yamazaki et al. (1996)
CYP3A4 Inhibition *(BFC substrate)*	Human recombinant (2.5 pmol/mL)	ketoconazole	Stresser et al. (2000)
CYP3A4 Inhibition *(BzRes substrate)*	Human recombinant (2.5 pmol/mL)	ketoconazole	Stresser et al. (2000)
	Human recombinant (2.5 pmol/mL)	ketoconazole	Lin et al. (2001)
	Human recombinant (22 pmol/mL)	ketoconazole	Nomeir et al. 2001
	Human recombinant (2.5 pmol/mL)	ketoconazole	Chang and Yeung (2001)
	Human liver microsomes [protein]=0.3 mg/mL	4 reference compounds (set 1)	Kuhnz and Gieschen (1998)

Notes:
CYP1A2: from PanVera, catalog number P2792.
CYP2B6: from Discovery Labware, catalog number 456255.
CYP2C9: from PanVera, catalog number P2378.
CYP2C19: from PanVera, catalog number P2570.
CYP2D6: from PanVera, catalog number P2283.
CYP2E1: from Discovery Labware, catalog number 456206.
CYP3A4: from PanVera, catalog number P2377.
CYP3A5: from PanVera, catalog number P2512.
4 Reference compounds - set 1: Propranolol, Imipramine, Verapamil, and Terfenadine.
Human liver microsomes: from Xenotech, catalog number: H0610, pooled and mixed gender.

2.6.2. Experimental Conditions

Assay	Substrate / Cofactor	Incubation	Detected Component	Analytical Method
	CEC (5 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 30 min, 37 °C	CHC	Fluorimetry
	EFC (1.5 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 50 min, 37 °C	HFC	Fluorimetry
	MFC (50 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 80 min, 37 °C	HFC	Fluorimetry
	CEC (25 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 60 min, 37 °C	CHC	Fluorimetry
	AMMC (1.5 µM), NADP (8.2 µM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 45 min, 37 °C	AHMC	Fluorimetry
	EC (4 µM), NADP (8.2 µM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 50 min, 37 °C	HC	Fluorimetry
	BFC (50 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 30 min, 37 °C	HFC	Fluorimetry
	BzRes (1 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 40 min, 37°C	resorufin	Fluorimetry
	Testosterone (50 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 15 min, 37°C	6β-hydroxy-testosterone	HPLC-UV/VIS
	Midazolam (5 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 20 min, 37°C	1-hydroxymidazolam	HPLC-UV/VIS

Assay	Substrate / Cofactor	Incubation	Detected Component	Analytical Method
	BFC (20 µM), NADP (1.3 mM), G6P (3.3 mM), G6PDHase (0.4 U/mL)	0 and 30 min, 37 °C	HFC	Fluorimetry
	Test compound (1 µM), NADP (1 mM), G6P (5 mM), G6PDHase (1 U/mL) with 0.6 % methanol, 0.6 % acetonitrile (n=2)	0 and 60 min, 37 °C Phosphate buffer (50 mM) pH 7.4	Product ion corresponding to the test compound via SRM	HPLC-MS/MS

Abbreviations:
AHMC: 3-[2-(N,N-diethyl-N-methylammonium)-ethyl]-7-hydroxy-4-methylcoumarin
AMMC: 3-[2-(N,N-diethyl-N-methylammonium)-ethyl]-7-methoxy-4-methylcoumarin; from Discovery Labware, catalog number 451700
BFC: 7-Benzyloxy-4-(trifluoromethyl)-coumarin; from Discovery Labware, catalog number 451730
BzRes : 7-benzyloxyresorufin
CEC: 3-Cyano-7-ethoxycoumarin, from Molecular Probes, catalog number C-684
CHC: 3-Cyano-7-hydroxycoumarin
CYP: Cytochrome P450
EC: 7-Ethoxycoumarin, from Molecular Probes, catalog number E-186
EFC: 7-Ethoxy-4-trifluoromethylcoumarin, from Molecular Probes, catalog number E-2882
G6P: D-Glucose-6-phosphate, from Sigma, catalog number G-7772
G6PDHase: Glucose-6-phosphate dehydrogenase, from Sigma, catalog number G-4134
HC: 7-Hydroxycoumarin
HFC: 7-Hydroxy-4-trifluoromethylcoumarin
HPLC-MS/MS: HPLC coupled with tandem mass spectrometry (Instrumentation: Thermo Finnigan)
HPLC: High performance liquid chromatography
MFC: 7-Methoxy-4-trifluoromethylcoumarin,from Sigma, catalog number T-3165
NADP: β-Nicotinamide adenine dinucleotide phosphate, from Sigma, catalog number N-0505
Res: Resorufin
SRM: Selected reaction monitoring

For CYP450 inhibition assays, the compound was tested at 10 µM concentration in duplicate as specified in the Results section of this report.

2.6.3. Analysis and Expression of Results

Cytochrome P450 Inhibition (fluorimetric detection)

The fluorescent intensity (*fu*) measured at (t = 0) was subtracted from that measured after the appropriate incubation time (t = final). The ratio of signal-to-noise was calculated by comparing the fluorescence in incubations containing the test compound to the control samples containing the same solvent vehicle. The percent of control activity was then calculated. Subsequently, the percent inhibition was calculated by subtracting the percent control activity from 100. IC_{50} values (concentration causing a half-maximal inhibition of control values) were determined by non-linear regression analysis of the concentration-response curves using Hill equation curve fitting.

Cytochrome P450 Inhibition (HPLC-UV/VIS and HPLC-MS/MS detection)

Peak areas corresponding to the metabolite of each substrate and the internal standard (when applicable) were recorded. The percent of control activity was then calculated. Subsequently, the percent inhibition was calculated by subtracting the percent control activity from 100 for each compound. IC_{50} values (concentration causing a half-maximal inhibition of control values) were determined by non-linear regression analysis of the concentration-response curves using Hill equation curve fitting.

Metabolic Stability

At the end of incubation at each of the time points, an equal volume of an organic mixture (acetonitrile/methanol, 50/50, v/v) containing an internal standard (when applicable) was added to the incubation mixture. Peak areas corresponding to the analytes were determined by HPLC-MS/MS. The ratio of precursor compound remaining after 60 minutes relative to the amount remaining at time zero, expressed as percent, is reported as metabolic stability.

2.7. ADME-Tox: Cytotoxicity

2.7.1. General Procedures

Assay	Tissue / Cell Source	Reference Compound	Bibliography
Cell viability *(HepG2)*	HepG2 cells	chlorpromazine	Nociari et al. (1998)

2.7.2. Experimental Conditions

Assay	Substrate / Stimulus	Incubation	Reaction Product	Analytical Method
	AlamarBlue oxidized (resazurin)	48 hours, 37 °C	AlamarBlue reduced (resorufin)	Fluorimetry

The compound was tested at 30 µM in duplicate with a final DMSO (dimethylsulfoxide) concentration of 1 %.

2.7.3. Analysis and Expression of Results

The percent of control activity was calculated. Subsequently, the percent of inhibition was calculated by subtraction of the percent of control value from 100. The IC_{50} value (concentration causing a half-maximal inhibition of control values) was determined by non-linear regression analysis of the concentration-response curve using Hill equation curve fitting.

3. COMPOUNDS

3.1. Test Compound

From: PFIZER GLOBAL RESEARCH & DEVELOPMENT

CEREP I.D.	Compound I.D.	Batch Number	Submitted M.W.	Stock Solution	Working Dilution
884017-11	PF-592379-00	11350201	235.33	1.E-02 M DMSO	1.E-04 M H2O*
				1.E-02 M DMSO	3.E-04 M H2O**
				1.E-02 M DMSO	5.E-05 M H2O***
				1.E-02 M DMSO	1.E-03 M H2O****

M.W.: Molecular Weight
* For *In Vitro* Pharmacology
** For the ATPase (Na^+/K^+) enzyme assay
*** For the human MMP-2, MMP-3 and MMP-9 assays and the Abl kinase assay
**** For final test concentrations higher than 1.E-05 M

3.2. Reference Compounds

In each experiment, the respective reference compounds were tested concurrently with PF-592379-00 in order to assess the assay suitability. It was tested either at one or several concentrations (for IC_{50} or EC_{50} value determination), and the data were compared with historical values determined at Cerep. The assay was rendered valid if the suitability criteria were met, in accordance with the corresponding Standard Operating Procedure.

4. RESULTS

4.1. *IN VITRO* PHARMACOLOGY: Binding Assays

The mean values for the effects of PF-592379-00 are summarized in table 1 - 1.
The individual data obtained with PF-592379-00 are reported in table 1 - 2.
The IC_{50} and K_i values for each reference compound are indicated in table 1 - 3. Each is within accepted limits of the historic average ± 0.5 log units.

The IC_{50} and K_i values determined for PF-592379-00 are indicated in table 1 - 4.
The corresponding competition curves obtained with PF-592379-00 are shown in figures 1 to 9.
The individual data obtained with PF-592379-00 are reported in table 1 - 5.
The IC_{50} and K_i values for each reference compound are indicated in table 1 - 6. Each is within accepted limits of the historic average ± 0.5 log units.

Table 1 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
A_1 *(h)* 884017-11	PF-592379-00	1.0E-05	-19
A_{2A} *(h)* 884017-11	PF-592379-00	1.0E-05	1
A_3 *(h)* 884017-11	PF-592379-00	1.0E-05	9
α_1 (non-selective) 884017-11	PF-592379-00	1.0E-05	-7
α_2 (non-selective) 884017-11	PF-592379-00	1.0E-05	16
α_{2A} *(h)* 884017-11	PF-592379-00	1.0E-05	3
α_{2B} 884017-11	PF-592379-00	1.0E-05	41
β_1 *(h)* 884017-11	PF-592379-00	1.0E-05	24
β_2 *(h)* 884017-11	PF-592379-00	1.0E-05	12
β_3 *(h)* 884017-11	PF-592379-00	1.0E-05	0
AT_1 *(h)* 884017-11	PF-592379-00	1.0E-05	2
AT_2 *(h)* 884017-11	PF-592379-00	1.0E-05	-9
BZD (central) 884017-11	PF-592379-00	1.0E-05	11
B_2 *(h)* 884017-11	PF-592379-00	1.0E-05	-1
CGRP *(h)* 884017-11	PF-592379-00	1.0E-05	-18
CB_1 *(h)* 884017-11	PF-592379-00	1.0E-05	-8
CB_2 *(h)* 884017-11	PF-592379-00	1.0E-05	-5
CCK_A *(h)* (CCK_1) 884017-11	PF-592379-00	1.0E-05	-16

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
CCK_B *(h)* (CCK_2) 884017-11	PF-592379-00	1.0E-05	-10
D1 *(h)* 884017-11	PF-592379-00	1.0E-05	2
D2S *(h)* 884017-11	PF-592379-00	1.0E-05	2
D3 *(h)* 884017-11	PF-592379-00	1.0E-05	**82**
D4.4 *(h)* 884017-11	PF-592379-00	1.0E-05	**80**
ET_B *(h)* 884017-11	PF-592379-00	1.0E-05	-4
$GABA_A$ 884017-11	PF-592379-00	1.0E-05	-18
$GABA_B$ 884017-11	PF-592379-00	1.0E-05	12
Kainate 884017-11	PF-592379-00	1.0E-05	-27
NMDA 884017-11	PF-592379-00	1.0E-05	3
Glycine (strychnine-insensitive) 884017-11	PF-592379-00	1.0E-05	-5
CCR1 *(h)* 884017-11	PF-592379-00	1.0E-05	-14
Ghrelin *(h)* (GHS) 884017-11	PF-592379-00	1.0E-05	7
H_1 (central) 884017-11	PF-592379-00	1.0E-05	47
H_2 884017-11	PF-592379-00	1.0E-05	10
H_3 884017-11	PF-592379-00	1.0E-05	24
I_1 (peripheral) 884017-11	PF-592379-00	1.0E-05	11
LTD_4 *(h)* 884017-11	PF-592379-00	1.0E-05	6
MC_1 884017-11	PF-592379-00	1.0E-05	12
MC_4 *(h)* 884017-11	PF-592379-00	1.0E-05	8

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
ML_1 884017-11	PF-592379-00	1.0E-05	-1
ML_2 *(MT_3)* 884017-11	PF-592379-00	1.0E-05	34
MAO-A 884017-11	PF-592379-00	1.0E-05	**70**
MAO-B 884017-11	PF-592379-00	1.0E-05	-16
M_1 *(h)* 884017-11	PF-592379-00	1.0E-05	-23
M_2 *(h)* 884017-11	PF-592379-00	1.0E-05	19
M_3 *(h)* 884017-11	PF-592379-00	1.0E-05	16
NK_1 *(h)* 884017-11	PF-592379-00	1.0E-05	-1
Y_1 *(h)* 884017-11	PF-592379-00	1.0E-05	10
N (neuronal) (α-BGTX-insensitive) 884017-11	PF-592379-00	1.0E-05	11
N *(h)* (muscle-type) 884017-11	PF-592379-00	1.0E-05	3
δ_2 *(h)* (DOP) 884017-11	PF-592379-00	1.0E-05	6
κ (KOP) 884017-11	PF-592379-00	1.0E-05	34
μ *(h)* (MOP) 884017-11	PF-592379-00	1.0E-05	4
ORL1 *(h)* (NOP) 884017-11	PF-592379-00	1.0E-05	-1
OT *(h)* 884017-11	PF-592379-00	1.0E-05	-32
PCP 884017-11	PF-592379-00	1.0E-05	0
P2X 884017-11	PF-592379-00	1.0E-05	5
$5\text{-}HT_{1A}$ *(h)* 884017-11	PF-592379-00	1.0E-05	39
$5\text{-}HT_{1B}$ 884017-11	PF-592379-00	1.0E-05	-5
$5\text{-}HT_{1D}$			

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
884017-11	PF-592379-00	1.0E-05	24
5-HT_{2A} *(h)* 884017-11	PF-592379-00	1.0E-05	8
5-HT_{2B} *(h)* 884017-11	PF-592379-00	1.0E-05	3
5-HT_{2C} *(h)* 884017-11	PF-592379-00	1.0E-05	32
5-HT_3 *(h)* 884017-11	PF-592379-00	1.0E-05	6
5-HT_{4e} *(h)* 884017-11	PF-592379-00	1.0E-05	-7
5-HT_6 *(h)* 884017-11	PF-592379-00	1.0E-05	25
5-HT_7 *(h)* 884017-11	PF-592379-00	1.0E-05	10
σ (non-selective) 884017-11	PF-592379-00	1.0E-05	7
sst_4 *(h)* 884017-11	PF-592379-00	1.0E-05	-3
sst_5 *(h)* 884017-11	PF-592379-00	1.0E-05	7
Glucocorticoid *(h)* (GR) 884017-11	PF-592379-00	1.0E-05	8
Estrogen α *(h)* (ERα) 884017-11	PF-592379-00	1.0E-05	-8
Androgen *(h)* (AR) 884017-11	PF-592379-00	1.0E-05	0
TH 884017-11	PF-592379-00	1.0E-05	-3
Urotensin-II (UT-II) 884017-11	PF-592379-00	1.0E-05	14
VIP_1 *(h)* ($VPAC_1$) 884017-11	PF-592379-00	1.0E-05	-5
V_{1a} *(h)* 884017-11	PF-592379-00	1.0E-05	22
Ca^{2+} channel (L, DHP site) 884017-11	PF-592379-00	1.0E-05	3
Ca^{2+} channel (L, verapamil site) (phenylalkylamines) 884017-11	PF-592379-00	1.0E-05	-6
Ryanodine (RY_3) 884017-11	PF-592379-00	1.0E-05	29

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
K^+_{ATP} channel 884017-11	PF-592379-00	1.0E-05	20
K^+_V channel 884017-11	PF-592379-00	1.0E-05	-3
SK^+_{Ca} channel 884017-11	PF-592379-00	1.0E-05	-11
Na^+ channel (site 2) 884017-11	PF-592379-00	1.0E-05	-35
Cl^- channel 884017-11	PF-592379-00	1.0E-05	-46
NE transporter *(h)* 884017-11	PF-592379-00	1.0E-05	1
DA transporter *(h)* 884017-11	PF-592379-00	1.0E-05	3
GABA transporter 884017-11	PF-592379-00	1.0E-05	-12
Choline transporter 884017-11	PF-592379-00	1.0E-05	-4
5-HT transporter *(h)* 884017-11	PF-592379-00	1.0E-05	16

Table 1 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	2nd	Mean
A_1 *(h)*					
884017-11	PF-592379-00	1.0E-05	115.1	122.6	**118.9**
A_{2A} *(h)*					
884017-11	PF-592379-00	1.0E-05	107.4	90.6	**99.0**
A_3 *(h)*					
884017-11	PF-592379-00	1.0E-05	93.9	88.5	**91.2**
α_1 (non-selective)					
884017-11	PF-592379-00	1.0E-05	109.2	105.4	**107.3**
α_2 (non-selective)					
884017-11	PF-592379-00	1.0E-05	92.7	75.6	**84.1**
α_{2A} *(h)*					
884017-11	PF-592379-00	1.0E-05	99.2	95.8	**97.5**
α_{2B}					
884017-11	PF-592379-00	1.0E-05	52.8	64.8	**58.8**
β_1 *(h)*					
884017-11	PF-592379-00	1.0E-05	85.9	66.5	**76.2**
β_2 *(h)*					
884017-11	PF-592379-00	1.0E-05	90.8	85.2	**88.0**
β_3 *(h)*					
884017-11	PF-592379-00	1.0E-05	102.3	97.5	**99.9**
AT_1 *(h)*					
884017-11	PF-592379-00	1.0E-05	98.4	97.0	**97.7**
AT_2 *(h)*					
884017-11	PF-592379-00	1.0E-05	111.6	105.7	**108.7**
BZD (central)					
884017-11	PF-592379-00	1.0E-05	88.8	89.4	**89.1**
B_2 *(h)*					
884017-11	PF-592379-00	1.0E-05	102.3	100.1	**101.2**
CGRP *(h)*					
884017-11	PF-592379-00	1.0E-05	112.5	123.1	**117.8**
CB_1 *(h)*					
884017-11	PF-592379-00	1.0E-05	101.1	115.5	**108.3**
CB_2 *(h)*					
884017-11	PF-592379-00	1.0E-05	99.6	111.4	**105.5**
CCK_A *(h)* (CCK_1)					
884017-11	PF-592379-00	1.0E-05	112.4	119.1	**115.7**

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
CCK_B *(h)* (CCK_2) 884017-11	PF-592379-00	1.0E-05	109.8	110.2	**110.0**
D1 *(h)* 884017-11	PF-592379-00	1.0E-05	97.1	99.7	**98.4**
D2S *(h)* 884017-11	PF-592379-00	1.0E-05	100.0	95.3	**97.6**
D3 *(h)* 884017-11	PF-592379-00	1.0E-05	17.7	19.3	**18.5**
D4.4 *(h)* 884017-11	PF-592379-00	1.0E-05	12.7	28.2	**20.4**
ET_B *(h)* 884017-11	PF-592379-00	1.0E-05	99.9	107.3	**103.6**
$GABA_A$ 884017-11	PF-592379-00	1.0E-05	124.1	111.1	**117.6**
$GABA_B$ 884017-11	PF-592379-00	1.0E-05	82.5	93.5	**88.0**
Kainate 884017-11	PF-592379-00	1.0E-05	152.5	101.0	**126.7**
NMDA 884017-11	PF-592379-00	1.0E-05	102.2	91.2	**96.7**
Glycine (strychnine-insensitive) 884017-11	PF-592379-00	1.0E-05	104.4	106.1	**105.2**
CCR1 *(h)* 884017-11	PF-592379-00	1.0E-05	111.4	116.9	**114.1**
Ghrelin *(h)* (GHS) 884017-11	PF-592379-00	1.0E-05	95.7	90.8	**93.3**
H_1 (central) 884017-11	PF-592379-00	1.0E-05	52.2	53.0	**52.6**
H_2 884017-11	PF-592379-00	1.0E-05	89.0	90.5	**89.7**
H_3 884017-11	PF-592379-00	1.0E-05	71.9	81.0	**76.5**
I_1 (peripheral) 884017-11	PF-592379-00	1.0E-05	79.4	98.6	**89.0**
LTD_4 *(h)* 884017-11	PF-592379-00	1.0E-05	79.7	107.4	**93.6**
MC_1 884017-11	PF-592379-00	1.0E-05	75.6	99.5	**87.6**
MC_4 *(h)* 884017-11	PF-592379-00	1.0E-05	91.7	92.5	**92.1**

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
ML_1 884017-11	PF-592379-00	1.0E-05	101.3	99.8	**100.6**
ML_2 *(MT3)* 884017-11	PF-592379-00	1.0E-05	64.9	67.5	**66.2**
MAO-A 884017-11	PF-592379-00	1.0E-05	31.9	28.0	**29.9**
MAO-B 884017-11	PF-592379-00	1.0E-05	115.7	116.8	**116.3**
M_1 *(h)* 884017-11	PF-592379-00	1.0E-05	116.0	130.0	**123.0**
M_2 *(h)* 884017-11	PF-592379-00	1.0E-05	77.8	83.3	**80.6**
M_3 *(h)* 884017-11	PF-592379-00	1.0E-05	91.9	75.5	**83.7**
NK_1 *(h)* 884017-11	PF-592379-00	1.0E-05	100.1	101.6	**100.8**
Y_1 *(h)* 884017-11	PF-592379-00	1.0E-05	81.2	98.3	**89.8**
N (neuronal) (α-BGTX-insensitive) 884017-11	PF-592379-00	1.0E-05	82.3	95.4	**88.9**
N *(h)* (muscle-type) 884017-11	PF-592379-00	1.0E-05	100.4	93.5	**97.0**
δ_2 *(h)* (DOP) 884017-11	PF-592379-00	1.0E-05	98.0	90.0	**94.0**
κ (KOP) 884017-11	PF-592379-00	1.0E-05	72.2	59.0	**65.6**
μ *(h)* (MOP) 884017-11	PF-592379-00	1.0E-05	84.7	106.4	**95.5**
ORL1 *(h)* (NOP) 884017-11	PF-592379-00	1.0E-05	100.1	100.9	**100.5**
OT *(h)* 884017-11	PF-592379-00	1.0E-05	147.5	117.4	**132.5**
PCP 884017-11	PF-592379-00	1.0E-05	106.2	94.3	**100.3**
P2X 884017-11	PF-592379-00	1.0E-05	92.5	97.6	**95.0**
$5\text{-}HT_{1A}$ *(h)* 884017-11	PF-592379-00	1.0E-05	62.6	60.3	**61.5**
$5\text{-}HT_{1B}$ 884017-11	PF-592379-00	1.0E-05	99.0	110.8	**104.9**
$5\text{-}HT_{1D}$					

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
884017-11	PF-592379-00	1.0E-05	72.4	78.7	**75.5**
5-HT_{2A} *(h)* 884017-11	PF-592379-00	1.0E-05	90.4	92.9	**91.6**
5-HT_{2B} *(h)* 884017-11	PF-592379-00	1.0E-05	99.9	93.2	**96.6**
5-HT_{2C} *(h)* 884017-11	PF-592379-00	1.0E-05	66.0	69.2	**67.6**
5-HT_3 *(h)* 884017-11	PF-592379-00	1.0E-05	97.3	91.1	**94.2**
5-HT_{4e} *(h)* 884017-11	PF-592379-00	1.0E-05	102.2	111.8	**107.0**
5-HT_6 *(h)* 884017-11	PF-592379-00	1.0E-05	77.3	73.3	**75.3**
5-HT_7 *(h)* 884017-11	PF-592379-00	1.0E-05	90.2	89.9	**90.0**
σ (non-selective) 884017-11	PF-592379-00	1.0E-05	99.8	87.2	**93.5**
sst_4 *(h)* 884017-11	PF-592379-00	1.0E-05	112.5	93.8	**103.1**
sst_5 *(h)* 884017-11	PF-592379-00	1.0E-05	87.8	97.4	**92.6**
Glucocorticoid *(h)* (GR) 884017-11	PF-592379-00	1.0E-05	103.9	81.1	**92.5**
Estrogen α *(h)* (ERα) 884017-11	PF-592379-00	1.0E-05	106.2	109.7	**108.0**
Androgen *(h)* (AR) 884017-11	PF-592379-00	1.0E-05	98.3	102.3	**100.3**
TH 884017-11	PF-592379-00	1.0E-05	102.1	103.2	**102.7**
Urotensin-II (UT-II) 884017-11	PF-592379-00	1.0E-05	91.5	80.9	**86.2**
VIP_1 *(h)* ($VPAC_1$) 884017-11	PF-592379-00	1.0E-05	103.5	105.6	**104.6**
V_{1a} *(h)* 884017-11	PF-592379-00	1.0E-05	75.8	81.2	**78.5**
Ca^{2+} channel (L, DHP site) 884017-11	PF-592379-00	1.0E-05	102.7	90.7	**96.7**
Ca^{2+} channel (L, verapamil site) (phenylalkylamines) 884017-11	PF-592379-00	1.0E-05	101.5	111.2	**106.3**
Ryanodine (RY_3) 884017-11	PF-592379-00	1.0E-05	58.3	83.9	**71.1**

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
K^+_{ATP} channel 884017-11	PF-592379-00	1.0E-05	85.3	74.9	**80.1**
K^+_V channel 884017-11	PF-592379-00	1.0E-05	103.5	102.1	**102.8**
SK^+_{Ca} channel 884017-11	PF-592379-00	1.0E-05	107.7	114.3	**111.0**
Na^+ channel (site 2) 884017-11	PF-592379-00	1.0E-05	120.2	150.4	**135.3**
Cl^- channel 884017-11	PF-592379-00	1.0E-05	150.5	141.0	**145.7**
NE transporter *(h)* 884017-11	PF-592379-00	1.0E-05	95.5	103.0	**99.3**
DA transporter *(h)* 884017-11	PF-592379-00	1.0E-05	100.2	94.7	**97.4**
GABA transporter 884017-11	PF-592379-00	1.0E-05	113.2	110.8	**112.0**
Choline transporter 884017-11	PF-592379-00	1.0E-05	91.1	117.2	**104.2**
5-HT transporter *(h)* 884017-11	PF-592379-00	1.0E-05	85.4	81.8	**83.6**

Table 1 - 3

Reference Compound Data

Assay Reference Compound	IC_{50} (nM)	K_i (nM)	nH
A_1 *(h)* DPCPX	2.3E-08	1.4E-08	*1.3*
A_{2A} *(h)* NECA	2.8E-08	2.3E-08	*0.7*
A_3 *(h)* IB-MECA	2.2E-09	1.5E-09	*0.9*
α_1 (non-selective) prazosin	6.4E-10	1.7E-10	*0.7*
α_2 (non-selective) yohimbine	7.7E-08	3.3E-08	*1.1*
α_{2A} *(h)* yohimbine	3.5E-09	1.5E-09	*1.2*
α_{2B} yohimbine	9.8E-09	3.8E-09	*0.7*
β_1 *(h)* atenolol	1.5E-06	6.7E-07	*0.8*
β_2 *(h)* ICI 118551	3.6E-09	1.5E-09	*1.2*
β_3 *(h)* cyanopindolol	5.3E-08	3.5E-08	*0.7*
AT_1 *(h)* saralasin	1.6E-09	1.2E-09	*1.3*
AT_2 *(h)* saralasin	1.5E-10	5.7E-11	*1.0*
BZD (central) diazepam	1.2E-08	9.9E-09	*0.9*
B_2 *(h)* NPC 567	6.6E-09	3.0E-09	*1.0*
CGRP *(h)* hCGRPα	1.8E-10	4.1E-11	*1.0*
CB_1 *(h)* WIN 55212-2	2.5E-08	1.8E-08	*1.1*
CB_2 *(h)* WIN 55212-2	4.2E-09	1.5E-09	*1.3*
CCK_A *(h)* (CCK_1) CCK-8	7.8E-10	4.8E-10	*0.6*
CCK_B *(h)* (CCK_2)			

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
CCK-8	2.1E-09	1.4E-09	*1.0*
D1 *(h)* SCH 23390	6.2E-10	2.9E-10	*0.9*
D2S *(h)* (+)butaclamol	5.9E-09	2.1E-09	*1.1*
D3 *(h)* (+)butaclamol	6.1E-08	1.3E-08	*1.3*
D4.4 *(h)* clozapine	1.3E-07	5.6E-08	*1.1*
ET_B *(h)* endothelin-3	1.3E-10	9.8E-11	*0.7*
$GABA_A$ muscimol	1.0E-08	7.2E-09	*1.2*
$GABA_B$ baclofen	5.7E-08	3.1E-08	*1.4*
Kainate kainic acid	3.2E-08	2.5E-08	*1.0*
NMDA CGS 19755	3.9E-07	3.2E-07	*1.2*
Glycine (strychnine-insensitive) glycine	4.4E-07	4.0E-07	*0.8*
CCR1 *(h)* MIP-1α	8.8E-11	3.2E-11	*1.1*
Ghrelin *(h)* (GHS) ghrelin	4.2E-10	1.7E-10	*1.3*
H_1 (central) pyrilamine	2.2E-09	9.5E-10	*1.3*
H_2 cimetidine	1.1E-06	9.1E-07	*0.7*
H_3 (R)α-Me-histamine	1.5E-09	6.0E-10	*0.7*
I_1 (peripheral) rilmenidine	2.3E-07	1.2E-07	*0.8*
LTD_4 *(h)* LTD_4	4.2E-09	2.8E-09	*0.7*
MC_1 NDP-α-MSH	9.2E-11	4.6E-11	*1.1*
MC_4 *(h)* NDP-α-MSH	3.0E-10	2.5E-10	*0.8*
ML_1 melatonin	2.1E-10	1.4E-10	*1.0*

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
ML_2 *(MT3)* melatonin	5.7E-08	5.6E-08	*0.9*
MAO-A clorgyline	1.6E-09	9.2E-10	*1.6*
MAO-B (R)-deprenyl	1.2E-08	6.8E-09	*0.9*
M_1 *(h)* pirenzepine	5.0E-08	4.3E-08	*0.7*
M_2 *(h)* methoctramine	4.8E-08	3.2E-08	*0.8*
M_3 *(h)* 4-DAMP	2.6E-09	1.9E-09	*0.8*
NK_1 *(h)* [Sar^9,$Met(O_2)^{11}$]-SP	3.1E-10	1.4E-10	*0.8*
Y_1 *(h)* NPY	2.2E-10	1.3E-10	*0.8*
N (neuronal) (α-BGTX-insensitive) nicotine	9.2E-09	5.0E-09	*0.9*
N *(h)* (muscle-type) α-bungarotoxin	7.2E-09	5.7E-09	*0.9*
δ_2 *(h)* (DOP) DPDPE	3.0E-09	1.8E-09	*1.1*
κ (KOP) U 50488	1.7E-09	5.6E-10	*1.4*
μ *(h)* (MOP) DAMGO	1.1E-09	3.8E-10	*0.8*
ORL1 *(h)* (NOP) nociceptin	6.3E-09	2.6E-09	*1.9*
OT *(h)* oxytocin	4.7E-07	4.6E-07	*1.2*
PCP MK 801	3.8E-09	3.6E-09	*1.0*
P2X α,β-MeATP	1.2E-08	5.5E-09	*0.9*
$5\text{-}HT_{1A}$ *(h)* 8-OH-DPAT	7.5E-10	3.8E-10	*1.1*
$5\text{-}HT_{1B}$ 5-HT	2.7E-08	1.7E-08	*0.7*
$5\text{-}HT_{1D}$ serotonin	1.2E-09	7.1E-10	*1.9*

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
5-HT_{2A} *(h)* ketanserin	2.5E-09	1.4E-09	*1.2*
5-HT_{2B} *(h)* serotonin	9.6E-08	4.3E-08	*1.0*
5-HT_{2C} *(h)* SB 242084	3.0E-08	1.4E-08	*2.2*
5-HT_3 *(h)* MDL 72222	2.9E-08	1.5E-08	*1.1*
5-HT_{4e} *(h)* 5-HT	1.9E-07	8.3E-08	*0.7*
5-HT_6 *(h)* serotonin	4.4E-07	2.1E-07	*0.8*
5-HT_7 *(h)* serotonin	5.0E-10	2.2E-10	*0.9*
σ (non-selective) haloperidol	7.8E-08	6.1E-08	*1.0*
sst_4 *(h)* somatostatin	1.7E-08	1.5E-08	*0.9*
sst_5 *(h)* somatostatin	1.2E-09	1.0E-09	*0.6*
Glucocorticoid *(h)* (GR) dexamethasone	6.0E-09	3.0E-09	*1.2*
Estrogen α *(h)* (ERα) 17-β-estradiol	4.5E-08	3.6E-08	*0.7*
Androgen *(h)* (AR) methyltrienolone	1.9E-09	1.5E-09	*1.2*
TH T_3	3.9E-10	2.8E-10	*1.0*
Urotensin-II (UT-II) urotensin-II	4.9E-09	4.5E-09	*0.9*
VIP_1 *(h)* ($VPAC_1$) VIP	2.9E-10	1.6E-10	*0.9*
V_{1a} *(h)* [$d(CH_2)_5^1$,Tyr$(Me)_2$]-AVP	1.2E-09	7.7E-10	*1.1*
Ca^{2+} channel (L, DHP site) nitrendipine	9.6E-10	3.2E-10	*1.3*
Ca^{2+} channel (L, verapamil site) (phenylalkylamines) D 600	1.0E-07	1.7E-08	*0.7*
Ryanodine (RY_3) ryanodine	3.1E-09	2.0E-09	*1.0*
K^+_{ATP} channel			

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
glibenclamide	3.6E-09	1.2E-09	*2.0*
K^+_V channel α–dendrotoxin	1.4E-09	1.1E-09	*3.3*
SK^+_{Ca} channel apamin	2.8E-11	1.8E-11	*1.2*
Na^+ channel (site 2) veratridine	7.7E-06	6.9E-06	*0.8*
Cl^- channel picrotoxinin	1.3E-07	1.1E-07	*1.3*
NE transporter *(h)* protriptyline	1.2E-08	9.7E-09	*1.1*
DA transporter *(h)* BTCP	2.2E-08	9.9E-09	*0.9*
GABA transporter nipecotic acid	6.8E-06	6.8E-06	*0.7*
Choline transporter hemicholinium-3	1.1E-08	7.5E-09	*1.1*
5-HT transporter *(h)* imipramine	4.9E-09	2.0E-09	*1.0*

Table 1 - 4

IC_{50} Determination: Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	IC_{50} (M)	K_i (M)	n_H	Flags
α_{2B}					
884017-11	PF-592379-00	1.0E-05	4.0E-06	*0.9*	
D3 *(h)*					
884017-11	PF-592379-00	1.2E-06	2.5E-07	*0.7*	
D4.4 *(h)*					
884017-11	PF-592379-00	6.6E-07	2.8E-07	*0.6*	
H_1 (central)					
884017-11	PF-592379-00	1.6E-05	6.9E-06	*0.6*	
ML_2 *(MT_3)*					
884017-11	PF-592379-00	4.3E-05	4.2E-05	*0.7*	
MAO-A					
884017-11	PF-592379-00				N.C.
κ (KOP)					
884017-11	PF-592379-00	9.5E-05	3.2E-05	*0.9*	
5-HT_{1A} *(h)*					
884017-11	PF-592379-00	1.9E-05	9.7E-06	*0.9*	
5-HT_{2C} *(h)*					
884017-11	PF-592379-00				N.C.

N.C.: Not calculable. IC50 value is not calculable because of less than 25% inhibition at the highest tested concentration.

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE ALPHA 2B RECEPTOR

IC50 = 1.0E-05 M
nH = 0.9

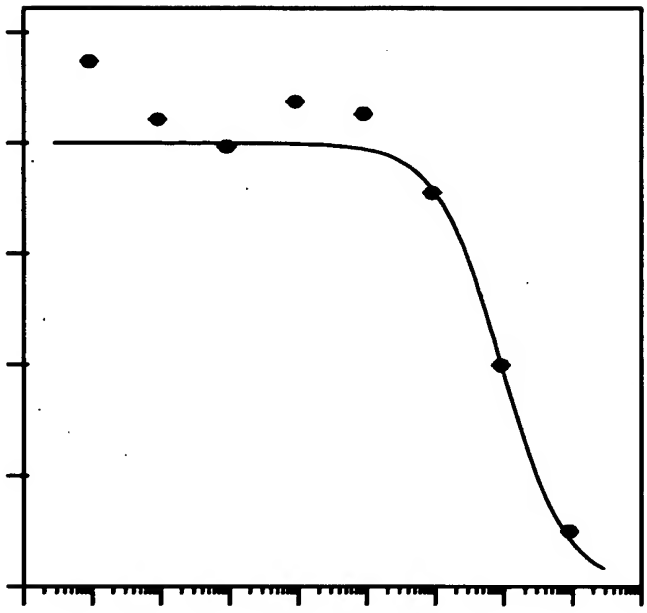


Figure 1

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE HUMAN D3 RECEPTOR

IC50 = 1.2E-06 M
nH = 0.7

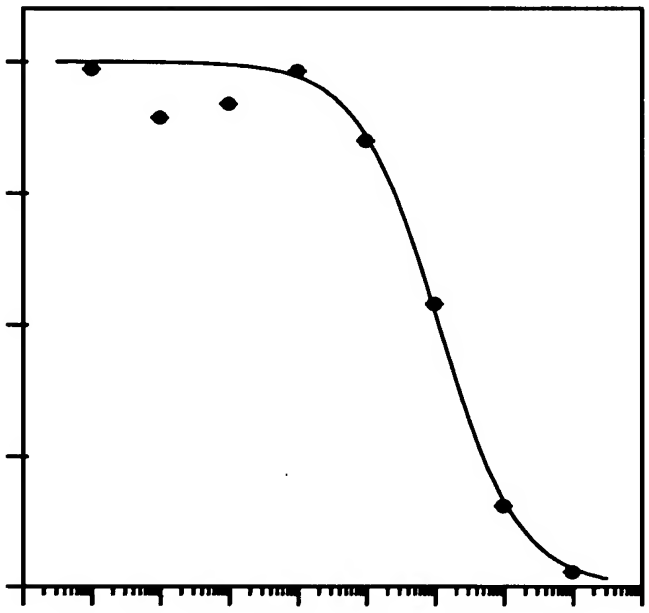


Figure 2

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE HUMAN D4.4 RECEPTOR

IC50 = 6.6E-07 M
nH = 0.6

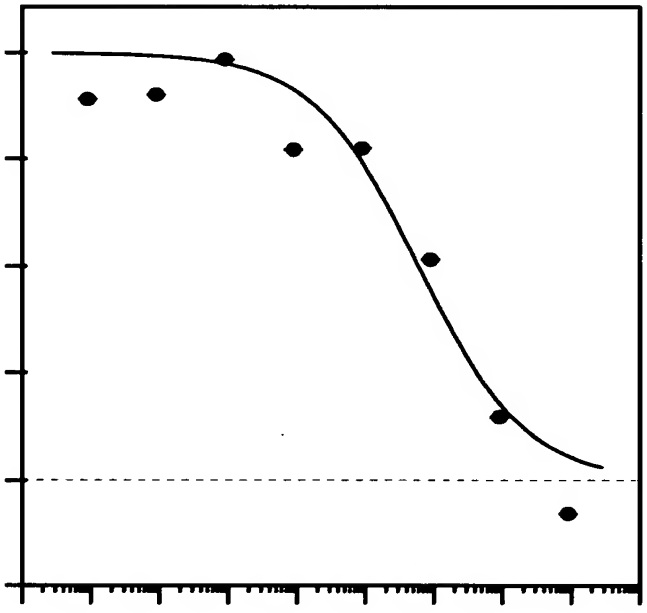


Figure 3

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE CENTRAL H1 RECEPTOR

IC50 = 1.6E-05 M
nH = 0.6

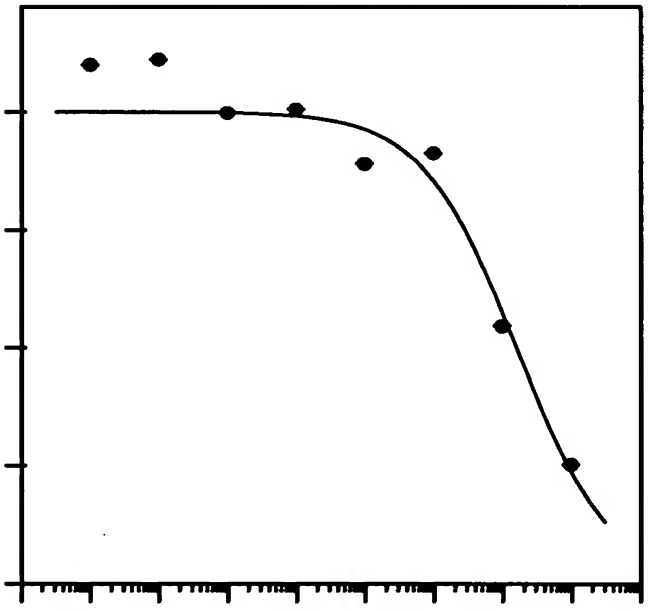


Figure 4

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE ML2 RECEPTOR

IC50 = 4.3E-05 M
nH = 0.7

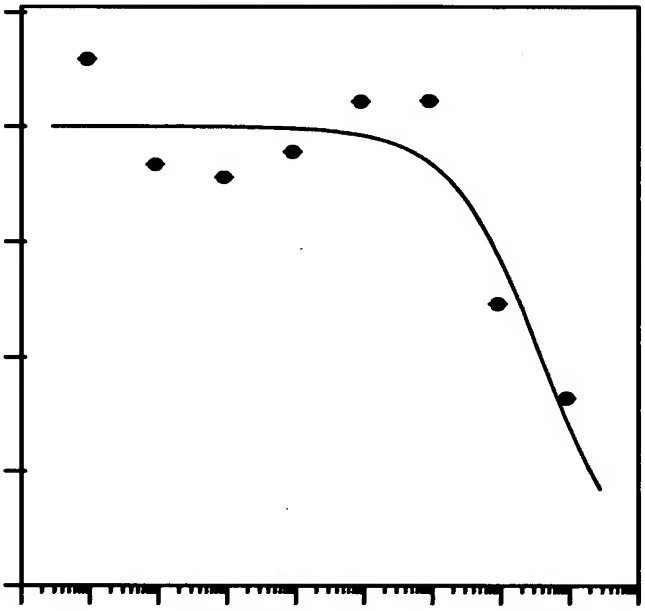


Figure 5

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE MAO-A RECEPTOR

IC50 not calculable

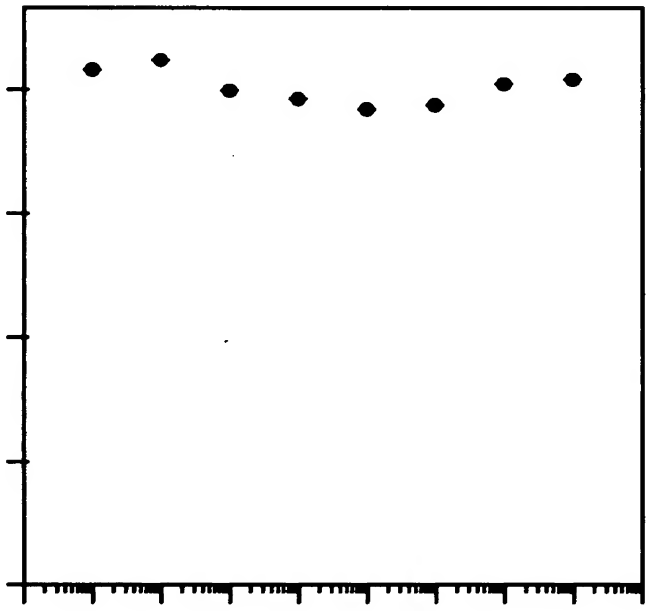


Figure 6

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE KAPPA RECEPTOR

IC50 = 9.5E-05 M
nH = 0.9

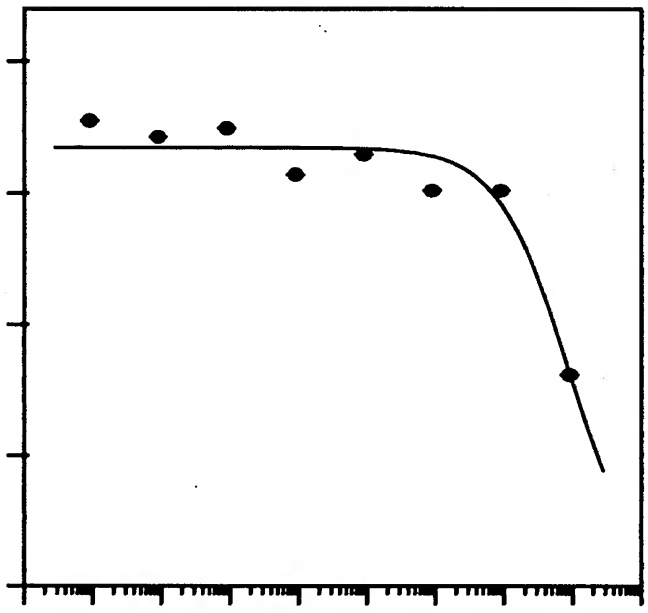


Figure 7

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE HUMAN 5-HT1A RECEPTOR

IC50 = 1.9E-05 M
nH = 0.9

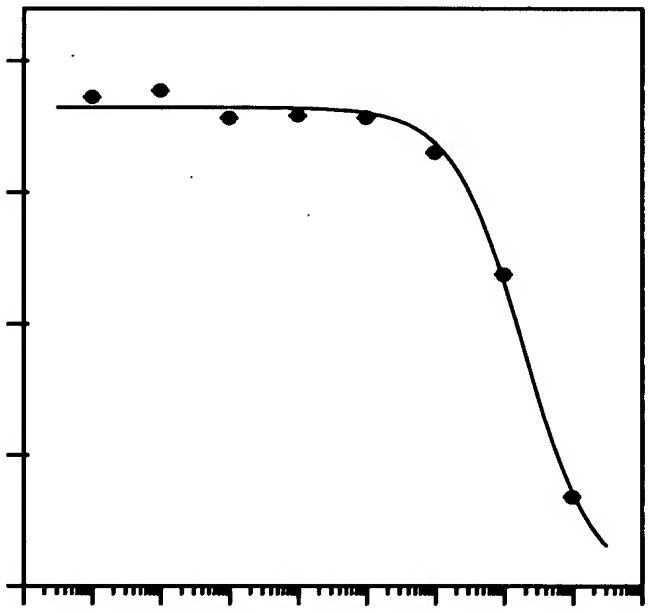


Figure 8

COMPETITION CURVE OBTAINED WITH PF-592379-00 AT THE HUMAN 5-HT2C RECEPTOR

IC50 not calculable

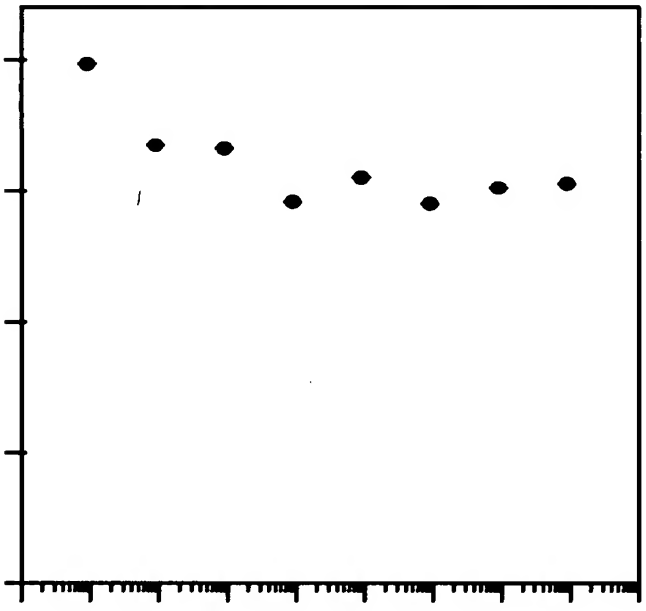


Figure 9

Table 1 - 5

IC_{50} Determination : Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd
α_{2B}							
884017-11	PF-592379-00	1.0E-11	104.3	132.6	**118.5**		
884017-11	PF-592379-00	1.0E-10	102.8	107.9	**105.4**		
884017-11	PF-592379-00	1.0E-09	93.8	104.6	**99.2**		
884017-11	PF-592379-00	1.0E-08	103.7	114.9	**109.3**		
884017-11	PF-592379-00	1.0E-07	103.7	109.4	**106.6**		
884017-11	PF-592379-00	1.0E-06	87.1	90.1	**88.6**		
884017-11	PF-592379-00	1.0E-05	73.3	26.5	**49.9**		
884017-11	PF-592379-00	1.0E-04	9.9	15.1	**12.5**		
D3 *(h)*							
884017-11	PF-592379-00	1.0E-11	95.2	102.1	**98.6**		
884017-11	PF-592379-00	1.0E-10	94.3	84.5	**89.4**		
884017-11	PF-592379-00	1.0E-09	86.6	97.5	**92.0**		
884017-11	PF-592379-00	1.0E-08	97.8	98.7	**98.2**		
884017-11	PF-592379-00	1.0E-07	83.5	86.5	**85.0**		
884017-11	PF-592379-00	1.0E-06	55.4	52.6	**54.0**		
884017-11	PF-592379-00	1.0E-05	15.9	15.1	**15.5**		
884017-11	PF-592379-00	1.0E-04	2.9	2.7	**2.8**		
D4.4 *(h)*							
884017-11	PF-592379-00	1.0E-11	83.2	94.8	**89.0**		
884017-11	PF-592379-00	1.0E-10	86.8	93.3	**90.1**		
884017-11	PF-592379-00	1.0E-09	102.7	94.1	**98.4**		
884017-11	PF-592379-00	1.0E-08	78.9	75.3	**77.1**		
884017-11	PF-592379-00	1.0E-07	71.7	83.2	**77.5**		
884017-11	PF-592379-00	1.0E-06	45.0	58.0	**51.5**		
884017-11	PF-592379-00	1.0E-05	11.9	17.7	**14.8**		
884017-11	PF-592379-00	1.0E-04	-9.0	-6.8	**-7.9**		
H_1 (central)							
884017-11	PF-592379-00	1.0E-11	107.6	112.4	**110.0**		
884017-11	PF-592379-00	1.0E-10	102.4	119.8	**111.1**		
884017-11	PF-592379-00	1.0E-09	99.4	100.2	**99.8**		
884017-11	PF-592379-00	1.0E-08	104.3	96.9	**100.6**		
884017-11	PF-592379-00	1.0E-07	82.4	95.7	**89.1**		
884017-11	PF-592379-00	1.0E-06	81.7	100.9	**91.3**		
884017-11	PF-592379-00	1.0E-05	48.7	60.6	**54.6**		
884017-11	PF-592379-00	1.0E-04	24.3	26.2	**25.3**		
ML_2 *(MT_3)*							
884017-11	PF-592379-00	1.0E-11	113.4	116.1	**114.7**		
884017-11	PF-592379-00	1.0E-10	86.4	97.2	**91.8**		

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd
884017-11	PF-592379-00	1.0E-09	87.9	90.0	**88.9**		
884017-11	PF-592379-00	1.0E-08	95.5	93.6	**94.5**		
884017-11	PF-592379-00	1.0E-07	103.6	107.2	**105.4**		
884017-11	PF-592379-00	1.0E-06	106.4	104.8	**105.6**		
884017-11	PF-592379-00	1.0E-05	61.5	32.8	**61.5**	{}	
884017-11	PF-592379-00	1.0E-04	37.7	44.6	**41.1**		
MAO-A							
884017-11	PF-592379-00	1.0E-11	100.4	107.9	**104.1**		
884017-11	PF-592379-00	1.0E-10	109.8	102.2	**106.0**		
884017-11	PF-592379-00	1.0E-09	97.5	102.1	**99.8**		
884017-11	PF-592379-00	1.0E-08	96.0	100.3	**98.2**		
884017-11	PF-592379-00	1.0E-07	96.6	95.7	**96.1**		
884017-11	PF-592379-00	1.0E-06	94.6	99.2	**96.9**		
884017-11	PF-592379-00	1.0E-05	101.2	101.3	**101.3**		
884017-11	PF-592379-00	1.0E-04	101.7	102.6	**102.1**		
κ (KOP)							
884017-11	PF-592379-00	1.0E-11	94.1	83.6	**88.8**		
884017-11	PF-592379-00	1.0E-10	84.7	86.9	**85.8**		
884017-11	PF-592379-00	1.0E-09	83.0	91.9	**87.4**		
884017-11	PF-592379-00	1.0E-08	91.3	65.9	**78.6**		
884017-11	PF-592379-00	1.0E-07	91.9	73.1	**82.5**		
884017-11	PF-592379-00	1.0E-06	67.0	84.1	**75.6**		
884017-11	PF-592379-00	1.0E-05	79.7	71.4	**75.6**		
884017-11	PF-592379-00	1.0E-04	37.7	43.2	**40.5**		
5-HT_{1A} *(h)*							
884017-11	PF-592379-00	1.0E-11	87.0	99.2	**93.1**		
884017-11	PF-592379-00	1.0E-10	84.7	104.0	**94.4**		
884017-11	PF-592379-00	1.0E-09	88.2	90.2	**89.2**		
884017-11	PF-592379-00	1.0E-08	87.3	91.9	**89.6**		
884017-11	PF-592379-00	1.0E-07	85.0	93.4	**89.2**		
884017-11	PF-592379-00	1.0E-06	78.0	87.0	**82.5**		
884017-11	PF-592379-00	1.0E-05	57.9	60.9	**59.4**		
884017-11	PF-592379-00	1.0E-04	16.8	17.1	**17.0**		
5-HT_{2C} *(h)*							
884017-11	PF-592379-00	1.0E-11	93.8	104.7	**99.2**		
884017-11	PF-592379-00	1.0E-10	95.6	72.0	**83.8**		
884017-11	PF-592379-00	1.0E-09	82.0	84.4	**83.2**		
884017-11	PF-592379-00	1.0E-08	75.3	70.5	**72.9**		
884017-11	PF-592379-00	1.0E-07	83.5	71.7	**77.6**		
884017-11	PF-592379-00	1.0E-06	75.6	69.6	**72.6**		
884017-11	PF-592379-00	1.0E-05	79.0	72.3	**75.6**		
884017-11	PF-592379-00	1.0E-04	70.7	82.1	**76.4**		

{}: That replicate was excluded from the calculation

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	2nd	Mean	Flags 1st	2nd

Table 1 - 6

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	K_i (M)	*n*$_H$
α_{2B} yohimbine	1.2E-08	4.6E-09	*1.8*
D3 *(h)* (+)butaclamol	8.7E-08	1.9E-08	*1.3*
D4.4 *(h)* clozapine	7.4E-08	3.1E-08	*1.3*
H_1 (central) pyrilamine	1.7E-09	7.3E-10	*0.9*
ML_2 *(MT_3)* melatonin	1.2E-07	1.2E-07	*0.8*
MAO-A clorgyline	2.0E-09	1.2E-09	*1.9*
κ (KOP) U 50488	7.2E-10	2.4E-10	*0.8*
5-HT_{1A} *(h)* 8-OH-DPAT	5.8E-10	2.9E-10	*1.0*
5-HT_{2C} *(h)* SB 242084	8.6E-08	4.1E-08	*1.5*

4.2. *IN VITRO* PHARMACOLOGY: Enzyme and Cell-based Assays

The mean values for the inhibitory effects of PF-592379-00 are summarized in tables 2 - 1 and 2 - 7.
The individual data obtained with PF-592379-00 are reported in tables 2 - 2 and 2 - 8.
The IC_{50} value for each reference compound is indicated in tables 2 - 3 and 2 - 9. Each is within accepted limits of the historic average ± 0.5 log units.

The mean values for the stimulatory effects of PF-592379-00 are summarized in table 2 - 4.
The individual data obtained with PF-592379-00 are reported in table 2 - 5.
The EC_{50} value for each reference compound is indicated in table 2 - 6. Each is within accepted limits of the historic average ± 0.5 log units.

Table 2 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
COX_2 *(h)* (isolated enzyme)			
884017-11	PF-592379-00	1.0E-05	19
inducible NOS (isol. enz/ spectrophoto.)			
884017-11	PF-592379-00	1.0E-05	-3
Phosphodiesterase 2 *(h)*			
884017-11	PF-592379-00	1.0E-05	-7
Phosphodiesterase 3 *(h)*			
884017-11	PF-592379-00	1.0E-05	13
Phosphodiesterase 4 *(h)*			
884017-11	PF-592379-00	1.0E-05	6
Phosphodiesterase 5 *(h)*			
884017-11	PF-592379-00	1.0E-05	24
Phosphodiesterase 6			
884017-11	PF-592379-00	1.0E-05	3
Phosphodiesterase 11 (h)- Pfizer			
884017-11	PF-592379-00	1.0E-05	-9
ACE *(h)* (recombinant)			
884017-11	PF-592379-00	1.0E-05	10
Elastase *(h)*			
884017-11	PF-592379-00	1.0E-05	-2
HIV-1 protease *(h)*			
884017-11	PF-592379-00	1.0E-05	4
Neutral endopeptidase *(h)*			
884017-11	PF-592379-00	1.0E-05	-5
MMP-1 *(h)*			
884017-11	PF-592379-00	1.0E-05	2
MMP-2 *(h)*			
884017-11	PF-592379-00	1.0E-05	3
MMP-3 *(h)*			
884017-11	PF-592379-00	1.0E-05	6
MMP-7 *(h)*			
884017-11	PF-592379-00	1.0E-05	3
MMP-9 *(h)*			
884017-11	PF-592379-00	1.0E-05	-26
Tryptase *(h)*			
884017-11	PF-592379-00	1.0E-05	0
Phosphatase 1B *(h)*			

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
884017-11	PF-592379-00	1.0E-05	6
Abl kinase 884017-11	PF-592379-00	1.0E-05	-5
CAM kinase II 884017-11	PF-592379-00	1.0E-05	-22
ERK_2 ($P42^{mapk}$) 884017-11	PF-592379-00	1.0E-05	-13
$p56^{lyn}$ kinase 884017-11	PF-592379-00	1.0E-05	9
$p55^{fyn}$ kinase 884017-11	PF-592379-00	1.0E-05	-5
ZAP70 kinase *(h)* 884017-11	PF-592379-00	1.0E-05	6
Acetylcholinesterase *(h)* 884017-11	PF-592379-00	1.0E-05	-4
Catechol- O-methyl transferase 884017-11	PF-592379-00	1.0E-05	11
GABA transaminase 884017-11	PF-592379-00	1.0E-05	8
ATPase (Na^+/K^+) 884017-11	PF-592379-00	3.0E-05	6

Table 2 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	 2nd	 Mean
COX_2 *(h)* (isolated enzyme)					
884017-11	PF-592379-00	1.0E-05	75.6	85.6	**80.6**
inducible NOS (isol. enz/ spectrophoto.)					
884017-11	PF-592379-00	1.0E-05	99.1	106.5	**102.8**
Phosphodiesterase 2 *(h)*					
884017-11	PF-592379-00	1.0E-05	106.2	108.7	**107.4**
Phosphodiesterase 3 *(h)*					
884017-11	PF-592379-00	1.0E-05	86.5	87.4	**87.0**
Phosphodiesterase 4 *(h)*					
884017-11	PF-592379-00	1.0E-05	97.9	90.1	**94.0**
Phosphodiesterase 5 *(h)*					
884017-11	PF-592379-00	1.0E-05	78.9	72.6	**75.8**
Phosphodiesterase 6					
884017-11	PF-592379-00	1.0E-05	98.2	96.5	**97.3**
Phosphodiesterase 11 (h)- Pfizer					
884017-11	PF-592379-00	1.0E-05	107.9	110.8	**109.3**
ACE *(h)* (recombinant)					
884017-11	PF-592379-00	1.0E-05	80.4	99.0	**89.7**
Elastase *(h)*					
884017-11	PF-592379-00	1.0E-05	103.6	99.7	**101.6**
HIV-1 protease *(h)*					
884017-11	PF-592379-00	1.0E-05	94.2	98.5	**96.3**
Neutral endopeptidase *(h)*					
884017-11	PF-592379-00	1.0E-05	109.4	101.6	**105.5**
MMP-1 *(h)*					
884017-11	PF-592379-00	1.0E-05	97.8	97.3	**97.5**
MMP-2 *(h)*					
884017-11	PF-592379-00	1.0E-05	95.8	98.4	**97.1**
MMP-3 *(h)*					
884017-11	PF-592379-00	1.0E-05	96.7	91.5	**94.1**
MMP-7 *(h)*					
884017-11	PF-592379-00	1.0E-05	93.8	100.1	**96.9**
MMP-9 *(h)*					
884017-11	PF-592379-00	1.0E-05	121.9	130.0	**126.0**
Tryptase *(h)*					
884017-11	PF-592379-00	1.0E-05	101.2	98.0	**99.6**
Phosphatase 1B *(h)*					

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	 2nd	 Mean
884017-11	PF-592379-00	1.0E-05	91.3	95.8	**93.6**
Abl kinase 884017-11	PF-592379-00	1.0E-05	102.9	106.3	**104.6**
CAM kinase II 884017-11	PF-592379-00	1.0E-05	110.1	133.3	**121.7**
ERK_2 ($P42^{mapk}$) 884017-11	PF-592379-00	1.0E-05	106.1	120.8	**113.4**
$p56^{lyn}$ kinase 884017-11	PF-592379-00	1.0E-05	102.1	80.7	**91.4**
$p55^{fyn}$ kinase 884017-11	PF-592379-00	1.0E-05	109.1	100.2	**104.6**
ZAP70 kinase *(h)* 884017-11	PF-592379-00	1.0E-05	103.4	84.4	**93.9**
Acetylcholinesterase *(h)* 884017-11	PF-592379-00	1.0E-05	103.1	104.6	**103.9**
Catechol- O-methyl transferase 884017-11	PF-592379-00	1.0E-05	90.4	87.7	**89.0**
GABA transaminase 884017-11	PF-592379-00	1.0E-05	89.2	94.8	**92.0**
ATPase (Na^+/K^+) 884017-11	PF-592379-00	3.0E-05	95.9	92.5	**94.2**

Table 2 - 3

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	nH
COX_2 *(h)* (isolated enzyme) NS 398	2.8E-05	*0.7*
inducible NOS (isol. enz/ spectrophoto.) 1400W	2.2E-08	*1.2*
Phosphodiesterase 2 *(h)* EHNA	5.6E-06	*0.5*
Phosphodiesterase 3 *(h)* milrinone	1.1E-07	*1.4*
Phosphodiesterase 4 *(h)* rolipram	1.2E-06	*1.0*
Phosphodiesterase 5 *(h)* dipyridamole	8.8E-07	*1.1*
Phosphodiesterase 6 zaprinast	5.5E-07	*1.0*
Phosphodiesterase 11 (h)- Pfizer dipyridamole	5.0E-07	*1.3*
ACE *(h)* (recombinant) captopril	5.8E-09	*0.9*
Elastase *(h)* 3',4'dichloroisocoumarin	6.6E-06	*1.3*
HIV-1 protease *(h)* pepstatin A	1.7E-06	*1.1*
Neutral endopeptidase *(h)* thiorphan	1.8E-09	*0.4*
MMP-1 *(h)* GM6001	2.3E-09	*1.3*
MMP-2 *(h)* GM6001	1.8E-09	*1.5*
MMP-3 *(h)* GM6001	1.0E-08	*1.1*
MMP-7 *(h)* GM6001	1.3E-08	*0.9*
MMP-9 *(h)* GM6001	5.3E-10	*1.3*
Tryptase *(h)* leupeptin	6.6E-07	*0.9*
Phosphatase 1B *(h)*		

Assay Reference Compound	IC_{50} (M)	n_H
Na_3VO_4	5.3E-07	*0.8*
Abl kinase staurosporine	3.9E-07	*2.4*
CAM kinase II staurosporine	2.5E-09	*1.7*
ERK_2 ($P42^{mapk}$) staurosporine	2.7E-06	*0.8*
$p56^{lyn}$ kinase staurosporine	3.5E-07	*0.6*
$p55^{fyn}$ kinase staurosporine	6.4E-08	*1.5*
ZAP70 kinase *(h)* staurosporine	8.2E-09	*1.5*
Acetylcholinesterase *(h)* neostigmine	3.5E-08	*1.1*
Catechol- O-methyl transferase Ro 41-0960	4.6E-08	*1.4*
GABA transaminase AoAA	1.4E-07	*1.2*
ATPase (Na^+/K^+) ouabain	2.9E-07	*1.4*

Table 2 - 4

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Stimulation Relative to Control
Guanylyl cyclase (basal)			
884017-11	PF-592379-00	1.0E-05	2
D4.4 receptor - G protein coupling *(h)* (agonist effect)			
884017-11	PF-592379-00	1.0E-07	**58**
884017-11	PF-592379-00	1.0E-06	**96**
884017-11	PF-592379-00	1.0E-05	**134**
884017-11	PF-592379-00	1.0E-04	**153**

Table 2 - 5

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Stimulation Relative to Control 1st	2nd	Mean
Guanylyl cyclase (basal)					
884017-11	PF-592379-00	1.0E-05	3.9	0.7	**2.3**
D4.4 receptor - G protein coupling *(h)* (agonist effect)					
884017-11	PF-592379-00	1.0E-07	68.1	48.8	**58.4**
884017-11	PF-592379-00	1.0E-06	97.2	94.9	**96.1**
884017-11	PF-592379-00	1.0E-05	147.0	120.8	**133.9**
884017-11	PF-592379-00	1.0E-04	146.3	158.9	**152.6**

Table 2 - 6

Reference Compound Data

Assay Reference Compound	EC_{50} (nM)	Hill
Guanylyl cyclase (basal) sodium nitroprusside	5.1E-06	*1.1*
D4.4 receptor - G protein coupling *(h)* (agonist effect) dopamine	1.2E-08	*0.5*

Table 2 - 7

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
D4.4 receptor - G protein coupling *(h)* (antagonist effect)			
884017-11	PF-592379-00	1.0E-07	-70
884017-11	PF-592379-00	1.0E-06	-56
884017-11	PF-592379-00	1.0E-05	-56
884017-11	PF-592379-00	1.0E-04	-32

Table 2 - 8

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	2nd	Mean
D4.4 receptor - G protein coupling *(h)* (antagonist effect)					
884017-11	PF-592379-00	1.0E-07	150.4	190.0	**170.2**
884017-11	PF-592379-00	1.0E-06	150.9	161.4	**156.1**
884017-11	PF-592379-00	1.0E-05	156.6	155.4	**156.0**
884017-11	PF-592379-00	1.0E-04	112.4	151.1	**131.8**

Table 2 - 9

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	n_H
D4.4 receptor - G protein coupling *(h)* (antagonist effect)		
spiperone	7.7E-09	*1.0*

4.3. ADME-Tox: Solution Properties

Aqueous Solubility:
The summary results obtained with PF-592379-00 are reported in table 3 - 1.
The individual data obtained with PF-592379-00 are reported in table 3 - 2.
The data obtained with the reference compounds are reported in table 3 - 3.

Partition Coefficient Log D:
The summary results obtained with PF-592379-00 are reported in table 3 - 4.
The individual data obtained with PF-592379-00 are reported in table 3 - 5.
The data obtained with the reference compounds are reported in table 3 - 6.

The chromatograms and UV/VIS spectra for PF-592379-00 are included in this report as Appendix A.

Table 3 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	Flags
Aqueous Solubility (PBS, pH 7.4)			
884017-11	PF-592379-00	2.0E-04	ND

ND Test compound was undetectable in the calibration sample. This could be due to 1) Test compound's chromophore was insufficient for PDA detection (most likely); 2) Test compound failed to elute within chromatographic run time (rare).

Table 3 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	Flags
Aqueous Solubility (PBS, pH 7.4)			
884017-11	PF-592379-00	2.0E-04	ND

ND Test compound was undetectable in the calibration sample. This could be due to 1) Test compound's chromophore was insufficient for PDA detection (most likely); 2) Test compound failed to elute within chromatographic run time (rare).

Table 3 - 3

Reference Compound Data

Assay Reference Compound	Test Concentration (µM)	Wavelength of Detection (nm)	Solubility 1st (µM)	Solubility 2nd (µM)	Solubility Mean (µM)	Chromatographic Purity (%)
Aqueous Solubility (PBS, pH 7.4)						
Diethylstilbestrol	2.0E-04	230	7.13	7.14	**7.1**	100
Diethylstilbestrol	2.0E-04	230	7.29	7.37	**7.3**	100
Haloperidol	2.0E-04	230	54.46	53.85	**54.2**	100
Haloperidol	2.0E-04	230	54.70	46.04	**50.4**	100
Ketoconazole	2.0E-04	230	136.81	130.87	**133.8**	100
Ketoconazole	2.0E-04	230	140.34	147.24	**143.8**	100
Metoprolol tartrate	2.0E-04	230	189.11	185.19	**187.2**	100
Metoprolol tartrate	2.0E-04	230	193.53	193.59	**193.6**	100
Phenytoin	2.0E-04	230	104.06	100.68	**102.4**	99
Phenytoin	2.0E-04	230	105.38	101.43	**103.4**	100
Rifampicin	2.0E-04	230	183.75	192.07	**187.9**	100
Rifampicin	2.0E-04	230	184.55	181.88	**183.2**	100
Simvastatin	2.0E-04	230	11.54	7.58	**9.6**	100
Tamoxifen	2.0E-04	230	2.24	0.41	**1.3**	100
Tamoxifen	2.0E-04	230	0.85	0.68	**0.8**	100

Table 3 - 4

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Flags
Partition Coefficient *(log D, n-octanol/PBS, pH 7.4)*		
884017-11	PF-592379-00	ND
Partition Coefficient *(log D, cyclohexane/PBS, pH 7.4)*		
884017-11	PF-592379-00	ND

ND Test compound was undetectable in the calibration sample. This could be due to 1) Test compound's chromophore was insufficient for PDA detection (most likely); 2) Test compound failed to elute within chromatographic run time (rare).

Table 3 - 5

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Flags
Partition Coefficient *(log D, n-octanol/PBS, pH 7.4)*		
884017-11	PF-592379-00	ND
Partition Coefficient *(log D, cyclohexane/PBS, pH 7.4)*		
884017-11	PF-592379-00	ND

ND Test compound was undetectable in the calibration sample. This could be due to 1) Test compound's chromophore was insufficient for PDA detection (most likely); 2) Test compound failed to elute within chromatographic run time (rare).

Table 3 - 6

Reference Compound Data

Assay Reference Compound	Test Concentration (M)	Weighed Average of Three Replicates
Partition Coefficient *(log D, n-octanol/PBS, pH 7.4)*		
Diethylstilbestrol	1.0E-04	4.58
Haloperidol	1.0E-04	2.76
Ketoconazole	1.0E-04	3.37
Metoprolol tartrate	1.0E-04	-0.41
Phenytoin	1.0E-04	2.33
Rifampicin	1.0E-04	1.28
Simvastatin	1.0E-04	4.46
Tamoxifen	1.0E-04	>4.5
Partition Coefficient *(log D, cyclohexane/PBS, pH 7.4)*		
Diethylstilbestrol	1.0E-04	1.73
Diethylstilbestrol	1.0E-04	1.88
Haloperidol	1.0E-04	0.39
Haloperidol	1.0E-04	0.37
Ketoconazole	1.0E-04	-0.17
Ketoconazole	1.0E-04	-0.20
Metoprolol tartrate	1.0E-04	-2.69
Metoprolol tartrate	1.0E-04	-2.04
Phenytoin	1.0E-04	0.69
Phenytoin	1.0E-04	-0.15
Rifampicin	1.0E-04	-1.21
Rifampicin	1.0E-04	-1.57
Simvastatin	1.0E-04	2.18
Simvastatin	1.0E-04	2.02
Tamoxifen	1.0E-04	>4.6
Tamoxifen	1.0E-04	>4.6

4.4. ADME-Tox: Bioanalytical

The individual data obtained with PF-592379-00 are reported in table 4 - 1.

The HPLC-MS total ion current chromatograms in positive and negative ionization modes, the full scan mass spectra, and the product ion spectra of PF-592379-00 obtained from HPLC-MS/MS screening are included in this report as Appendix B.

Table 4 - 1

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Molecular Weight	FW	Selected ESI (+) Precursor Ion (m/z)	Product Ion (m/z)	Collision Offset (V)	Ionization Classification
HPLC-MS Screen							
884017-11	PF-592379-00	235.33	235.33	236.3	121.0	-30	2.0

Notes:
Ionization Classification:
1 = Highly ionizable compound
2 = Intermediately ionizable compound
3 = Poorly ionizable compound

4.5. ADME-Tox: *In Vitro* Absorption

Permeability:
The summary results obtained with PF-592379-00 are reported in table 5 - 1.
The individual data obtained with PF-592379-00 are reported in table 5 - 2.
The data obtained with the reference compounds are reported in table 5 - 3.

P-glycoprotein Inhibition:
The mean values for the effects PF-592379-00 are summarized in table 5 - 4.
The individual data obtained with PF-592379-00 are reported in table 5 - 5.
The IC_{50} value for the reference compound is indicated in table 5 - 6.

Table 5 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	Mean TC7 Permeability (10^{-6} cm/s)	Flags	Mean Recovery (%)
A-B Permeability *(pH 6.5/7.4)*					
884017-11	PF-592379-00	5.0E-05		ND	
A-B Permeability *(pH 7.4/7.4)*					
884017-11	PF-592379-00	5.0E-05		ND	
B-A Permeability *(pH 6.5/7.4)*					
884017-11	PF-592379-00	5.0E-05	25.2		101
B-A Permeability *(pH 7.4/7.4)*					
884017-11	PF-592379-00	5.0E-05	20.5		94

ND Test compound was not detected in the assay matrix.

Table 5 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	TC7 Permeability 1^{st} (10^{-6} cm/s)	TC7 Permeability 2^{nd} (10^{-6} cm/s)	TC7 Permeability Mean (10^{-6} cm/s)	Flags	Percent Recovery 1^{st} (%)	Percent Recovery 2^{nd} (%)	Percent Recovery Mean (%)
A-B Permeability *(pH 6.5/7.4)*									
884017-11	PF-592379-00	5.0E-05				ND			
A-B Permeability *(pH 7.4/7.4)*									
884017-11	PF-592379-00	5.0E-05				ND			
B-A Permeability *(pH 6.5/7.4)*									
884017-11	PF-592379-00	5.0E-05	25.52	24.89	**25.2**		106	96	**101**
B-A Permeability *(pH 7.4/7.4)*									
884017-11	PF-592379-00	5.0E-05	19.14	21.78	**20.5**		94	94	**94**

ND Test compound was not detected in the assay matrix.

Table 5 - 3

Reference Compound Data

Assay Reference Compound	Test Concentration (M)	TC7 Permeability 1st (10^{-6} cm/s)	TC7 Permeability 2nd (10^{-6} cm/s)	TC7 Permeability Mean (10^{-6} cm/s)	Percent Recovery 1st (%)	Percent Recovery 2nd (%)	Percent Recovery Mean (%)
A-B Permeability *(pH 6.5/7.4)*							
Propranolol	5.0E-05	36.29	38.42	**37.4**	91	86	**89**
Ranitidine	5.0E-05	0.53	0.64	**0.6**	85	84	**85**
Vinblastine	5.0E-05	1.68	3.39	**2.5**	106	99	**103**
A-B Permeability *(pH 7.4/7.4)*							
Propranolol	5.0E-05	44.77	49.05	**46.9**	76	81	**78**
propranolol	5.0E-05	51.48	71.18	**61.3**	85	91	**88**
Ranitidine	5.0E-05	0.66	{3.96}	**0.7**	97	{98}	**97**
ranitidine	5.0E-05	0.99	1.03	**1.0**	53	77	**65**
Vinblastine	5.0E-05	0.06	0.49	**0.3**	81	103	**92**
vinblastine	5.0E-05	3.20	4.77	**4.0**	89	101	**95**
B-A Permeability *(pH 6.5/7.4)*							
propranolol	5.0E-05	22.43	20.79	**21.6**	69	88	**78**
ranitidine	5.0E-05	3.91	3.42	**3.7**	79	76	**77**
vinblastine	5.0E-05	37.49	44.41	**40.9**	71	101	**86**

Note : The data point in the brackets was excluded for the calculation of the mean value.

Table 5 - 4

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
P-glycoprotein Inhibition 884017-11	PF-592379-00	5.0E-05	13

Table 5 - 5

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	2nd	Mean
P-glycoprotein Inhibition 884017-11	PF-592379-00	5.0E-05	87.0	87.7	**87.4**

Table 5 - 6

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	n_H
P-glycoprotein Inhibition		
verapamil	3.0E-06	*0.6*

4.6. ADME-Tox: *In Vitro* Metabolism

Metabolic Stability:
The summary results obtained with PF-592379-00 are reported in table 6 - 1.
The individual data obtained with PF-592379-00 are reported in table 6 - 2.
The data obtained with the reference compounds are reported in table 6 - 3.

CYP Inhibition:
The mean values for the effects of PF-592379-00 are summarized in table 6 - 4.
The individual data obtained with PF-592379-00 are reported in table 6 - 5.
The IC_{50} values for the reference compounds are reported in table 6 - 6.

Table 6 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	Flags
Metabolic Stability (liver micros. *human*)			
884017-11	PF-592379-00	1.0E-06	ND

ND Test compound was not detected in the assay matrix.

Table 6 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	Flags
Metabolic Stability (liver micros. *human*)			
884017-11	PF-592379-00	1.0E-06	ND

ND Test compound was not detected in the assay matrix.

Table 6 - 3

Reference Compound Data

Assay Reference Compound	Test Concentration (M)	Parent Remaining 1st (%)	2nd (%)	Mean (%)
Metabolic Stability (liver micros. *human*)				
Imipramine	1.0E-06	91.7	85.1	**88**
Propranolol	1.0E-06	78.9	75.5	**77**
Terfenadine	1.0E-06	9.3	6.2	**8**
Verapamil	1.0E-06	12.7	12.7	**13**

Table 6 - 4

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
CYP1A2 Inhibition *(CEC substrate)*			
884017-11	PF-592379-00	1.0E-05	-9
CYP2B6 Inhibition *(EFC substrate)*			
884017-11	PF-592379-00	1.0E-05	16
CYP2C9 Inhibition *(7-MFC substrate)*			
884017-11	PF-592379-00	1.0E-05	15
CYP2C19 Inhibition *(CEC substrate)*			
884017-11	PF-592379-00	1.0E-05	6
CYP2D6 Inhibition *(AMMC substrate)*			
884017-11	PF-592379-00	1.0E-05	2
CYP2E1 Inhibition *(7-EC substrate)*			
884017-11	PF-592379-00	1.0E-05	0
CYP3A4 Inhibition *(BFC substrate)*			
884017-11	PF-592379-00	1.0E-05	7
CYP3A4 Inhibition *(BzRes substrate)*			
884017-11	PF-592379-00	1.0E-05	10
CYP3A4 Inhibition *(Testosterone substrate)*			
884017-11	PF-592379-00	1.0E-05	10
CYP3A4 Inhibition *(Midazolam substrate)*			
884017-11	PF-592379-00	1.0E-05	-3
CYP3A5 Inhibition *(BFC substrate)*			
884017-11	PF-592379-00	1.0E-05	9

Table 6 - 5

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	2nd	Mean
CYP1A2 Inhibition *(CEC substrate)*					
884017-11	PF-592379-00	1.0E-05	109.4	107.7	**108.6**
CYP2B6 Inhibition *(EFC substrate)*					
884017-11	PF-592379-00	1.0E-05	82.7	85.8	**84.3**
CYP2C9 Inhibition *(7-MFC substrate)*					
884017-11	PF-592379-00	1.0E-05	75.8	94.6	**85.2**
CYP2C19 Inhibition *(CEC substrate)*					
884017-11	PF-592379-00	1.0E-05	93.0	94.8	**93.9**
CYP2D6 Inhibition *(AMMC substrate)*					
884017-11	PF-592379-00	1.0E-05	98.1	97.3	**97.7**
CYP2E1 Inhibition *(7-EC substrate)*					
884017-11	PF-592379-00	1.0E-05	98.8	101.9	**100.4**
CYP3A4 Inhibition *(BFC substrate)*					
884017-11	PF-592379-00	1.0E-05	93.6	93.0	**93.3**
CYP3A4 Inhibition *(BzRes substrate)*					
884017-11	PF-592379-00	1.0E-05	89.6	89.9	**89.8**
CYP3A4 Inhibition *(Testosterone substrate)*					
884017-11	PF-592379-00	1.0E-05	90.5	89.9	**90.2**
CYP3A4 Inhibition *(Midazolam substrate)*					
884017-11	PF-592379-00	1.0E-05	104.7	101.4	**103.1**
CYP3A5 Inhibition *(BFC substrate)*					
884017-11	PF-592379-00	1.0E-05	92.1	90.6	**91.4**

Table 6 - 6

Reference Compound Data

Assay Reference Compound	IC_{50} (uM)	n H
CYP1A2 Inhibition *(CEC substrate)*		
furafylline	2.2E-06	*0.8*
CYP2B6 Inhibition *(EFC substrate)*		
ketoconazole	1.5E-05	*1.2*
CYP2C9 Inhibition *(7-MFC substrate)*		
sulfaphenazole	1.2E-07	*1.0*
CYP2C19 Inhibition *(CEC substrate)*		
tranylcypromine	3.5E-06	*1.0*
tranylcypromine	3.4E-06	*1.0*
CYP2D6 Inhibition *(AMMC substrate)*		
quinidine	1.3E-08	*1.3*
CYP2E1 Inhibition *(7-EC substrate)*		
4-methylpyrazole	3.5E-06	*0.9*
CYP3A4 Inhibition *(BFC substrate)*		
ketoconazole	1.7E-06	*1.0*
CYP3A4 Inhibition *(BzRes substrate)*		
ketoconazole	1.1E-06	*1.3*
CYP3A4 Inhibition *(Testosterone substrate)*		
ketoconazole	9.4E-07	*1.7*
CYP3A4 Inhibition *(Midazolam substrate)*		
ketoconazole	1.1E-06	*3.0*
CYP3A5 Inhibition *(BFC substrate)*		
ketoconazole	7.8E-07	*1.4*

4.7. ADME-Tox: Cytotoxicity

Cell Viability:

The mean values for the effects of PF-592379-00 are summarized in table 7 - 1.

The individual data obtained with PF-592379-00 are reported in table 7 - 2.

The IC_{50} value for the reference compound is indicated in table 7 - 3.

Table 7 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
Cell viability *(HepG2)* 884017-11	PF-592379-00	3.0E-05	3

Table 7 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	2nd	Mean
Cell viability *(HepG2)* 884017-11	PF-592379-00	3.0E-05	100.5	94.1	**97.3**

Table 7 - 3

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	nH
Cell viability *(HepG2)* chlorpromazine	1.8E-05	*2.9*

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6. STORAGE AND RETENTION OF RECORDS

All documents generated during the performance of the study (raw data, various recordings such as QA audit reports, an original of the study report, study plan...) will be stored for a 10-year period in Cerep's archive rooms after achievement of the study. Only Cerep's authorized employees shall have access to the archives.

The original final report provided to the sponsor will be kept by the sponsor under its sole responsibility.

7. QUALITY ASSURANCE STATEMENT

The following audits were performed on this study:

	CALENDAR
Audit of Raw Data	For each assay
Audits of the Final Report	

Audit reports were established for each audit performed.
Audit report of the study report was transmitted to the Study Director for approval.

I certify that results presented in this report were generated using the materials and methods mentioned and that these results accurately reflect the Raw Data.

Quality Unit

APPENDIX A

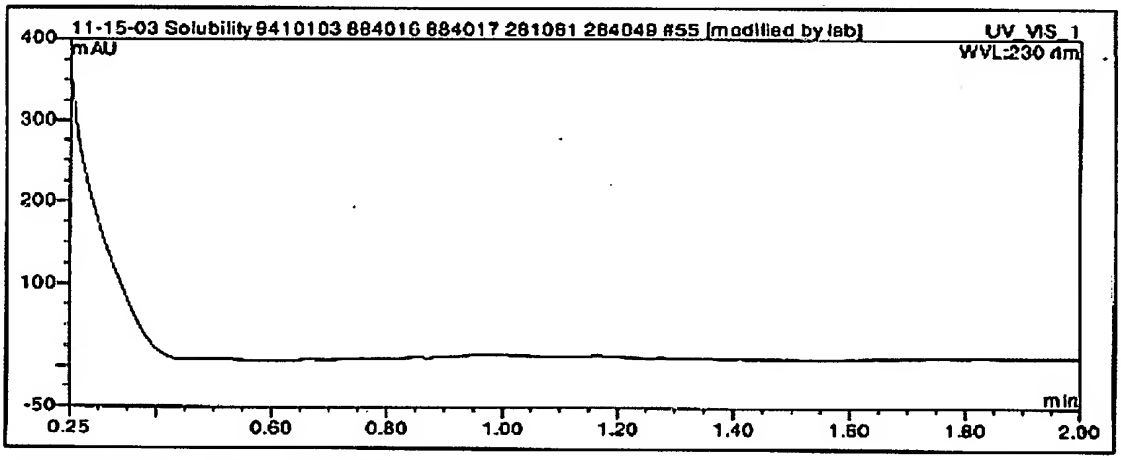
HPLC Chromatograms and UV/VIS Spectra of the Test Compound, Derived from the Aqueous Solubility Assay

Version 1
August 12, 2004

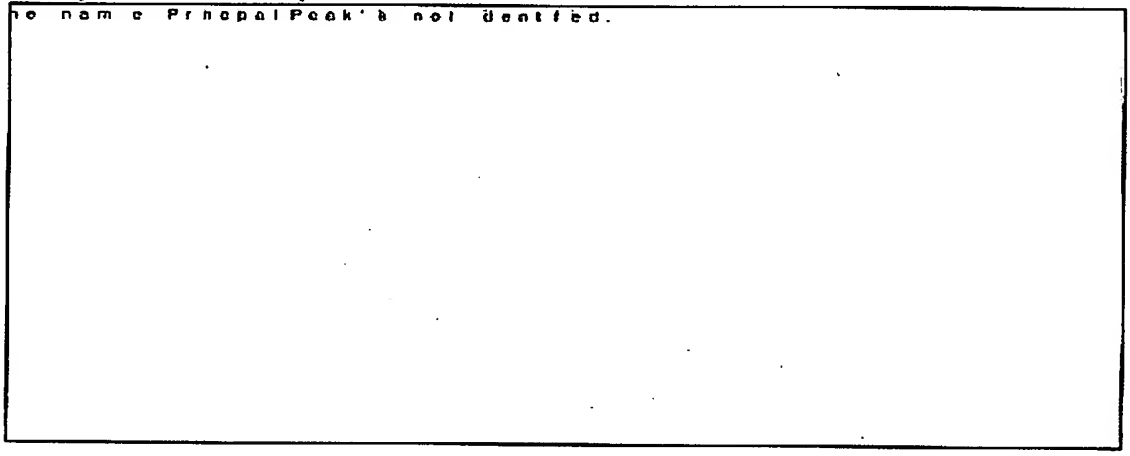
Cerep		Cal 884017-11	

Injection #	55	Reference Wavelength (nm)	550
Plate Position	Tray1	Retention Time of Principal Peak	n.a.
Well Position	30	Height of Principal Peak (mAU)	n.a.
Time Injected	11/15/03 14:52:31	Relative Area (Purity; %)	n.a.
Injection Volume	15	Relative Absorbance Maximum (nm)	n.a.
Wavelength (nm)	230	Absolute Absorbance Maximum (nm)	n.a.
Bandwidth (nm)	15	Peak Purity Match (max=1000)	n.a.

Chromatogram



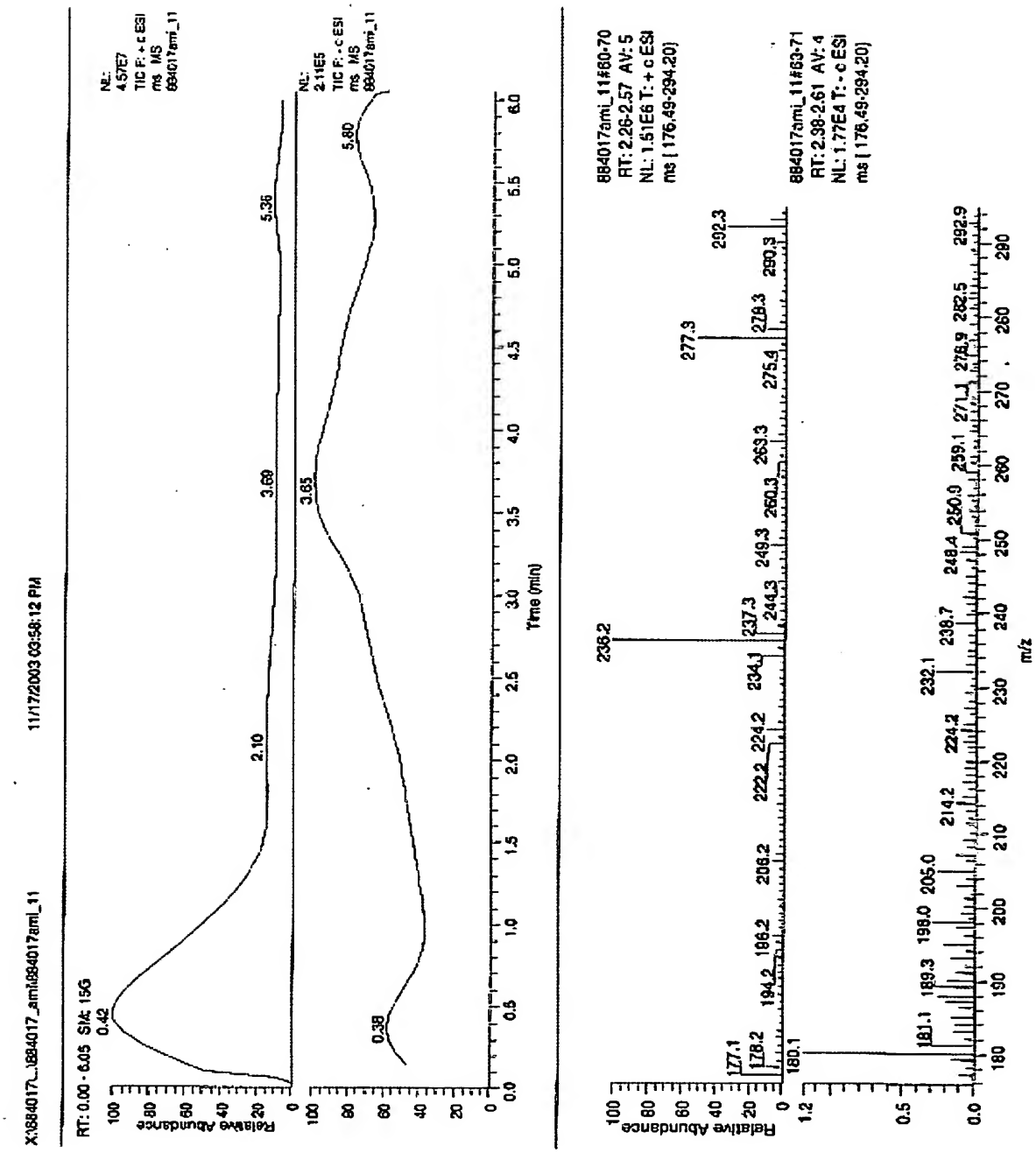
UV Spectrum of Principal Peak

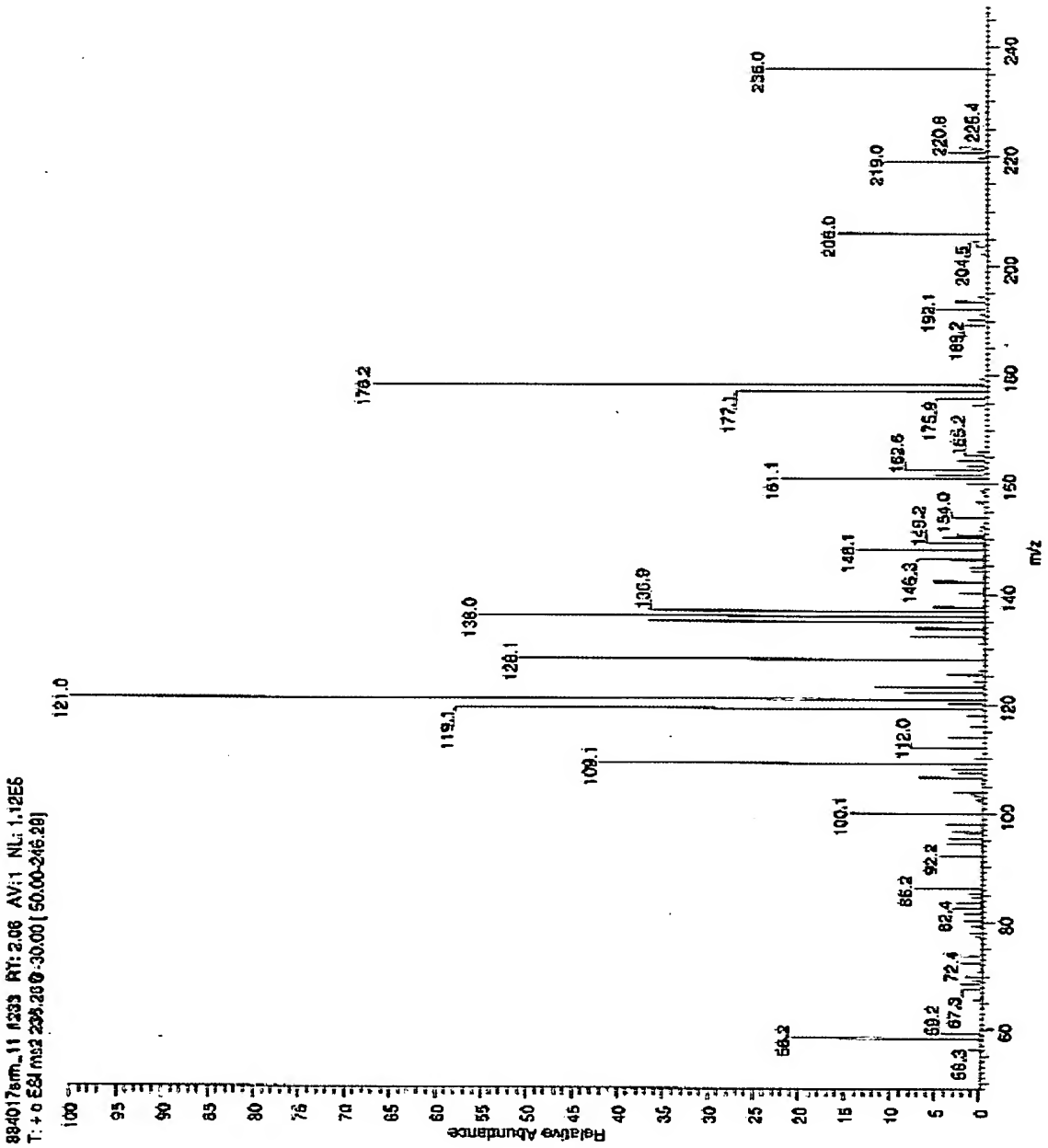


APPENDIX B

HPLC-MS Total Ion Current Chromatograms, Full Scan Mass Spectra, and Product Ion Mass Spectra of the Test Compound

Version 1
August 12, 2004





Appendix 2

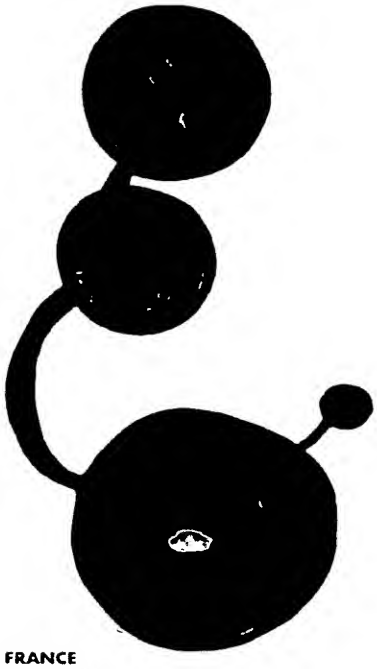
GLP Study report for binding assay 1 for Merck Example 3 enantiomer 1, completed at CEREP Biosciences

In this report, enantiomer 1 of Merck Example 3 is referred to by the reference number PF-4542563.

Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

Cerep

STUDY NUMBER 7570671b
FINAL REPORT



In Vitro Pharmacology: Pfizer Tier 0 Profile

- Study of PF-04542563-00 -

Annie OTTO-BRUC, Ph.D.

I FRANCE
le Bois l'Evêque
86600 CELLE L'EVESCAULT
Tel. +33 (0)5 49 89 30 00
Fax +33 (0)5 49 43 21 70

(Headquarters)
155 boulevard Haussmann
75008 PARIS
Tel. +33 (0)1 45 64 44 60
Fax +33 (0)1 45 64 44 64

I USA
15318 N.E. 95th Street
REDMOND WA 98052
Tel. +1 425 895 8666
Fax +1 425 895 8668

I www.cerep.com

I customer_support@cerep.fr

Cerep

Le Bois l'Evêque
B.P.1
86 600 Celle l'Evescault
France

www.cerep.com
Tel: 33 (0)5.49.89.30.00
Fax: 33 (0)5.49.43.21.70

Ref.: Final Report 7570671b/MN

STUDY NUMBER 7570671b

In Vitro Pharmacology: Pfizer Tier 0 Profile
- Study of PF-04542563-00 -

Study Sponsor:	PFIZER Limited
Attention:	Dr. Gareth WALDRON Senior Principal Scientist
Address:	Global Safety Pharmacology Ramsgate Road IPC 388 SANDWICH Kent CT13 9NJ UNITED KINGDOM
Study Director:	Annie OTTO-BRUC, Ph.D.
Testing Facility:	Cerep Le Bois l'Evêque - B.P. 1 - 86600 CELLE L'EVESCAULT, FRANCE
Study Period:	From March 06, 2007 to March 14, 2007
Report Version:	1
Report Date:	March 26, 2007

STUDY DIRECTOR

Annie OTTO-BRUC, Ph.D.
Principal Scientist, Pharmacology

March 26, 2007
Date

CONTENTS

1. PURPOSE OF THE STUDY

The purpose of this study was to investigate the effects of PF-04542563-00 in various *in vitro* receptor binding and enzyme assays.

2. MATERIALS AND METHODS

2.1. *IN VITRO* PHARMACOLOGY: Binding Assays

2.1.1. General Procedures

Assay	Origin	Reference Compound	Bibliography
A_1 *(h)*	human recombinant (CHO cells)	DPCPX	Townsend-Nicholson and Schofield (1994)
A_{2A} *(h)*	human recombinant (HEK-293 cells)	NECA	Luthin et al. (1995)
α_1 (non-selective)	rat cerebral cortex	prazosin	Greengrass and Bremner (1979)
α_{2A} *(h)*	human recombinant (CHO cells)	yohimbine	Langin et al. (1989)
α_{2B} *(h)*	human recombinant (CHO cells)	yohimbine	Devedjian et al. (1994)
β_1 *(h)*	human recombinant (HEK-293 cells)	atenolol	Levin et al. (2002)
β_2 *(h)*	human recombinant (Sf9 cells)	ICI 118551	Smith and Teitler (1999)
AT_1 *(h)*	human recombinant (HEK-293 cells)	saralasin	Le et al. (2005)
BZD (central)	rat cerebral cortex	diazepam	Speth et al. (1979)
CB_1 *(h)*	human recombinant (CHO cells)	CP 55940	Rinaldi-Carmona et al. (1996)
CB_2 *(h)*	human recombinant (CHO cells)	WIN 55212-2	Munro et al. (1993)
CCK_A *(h)* (CCK_1)	human recombinant (CHO cells)	CCK-8	Bignon et al. (1999)
CCK_B *(h)* (CCK_2)	human recombinant (CHO cells)	CCK-8	Lee et al. (1993)
D_1 *(h)*	human recombinant (CHO cells)	SCH 23390	Zhou et al. (1990)
D_{2S} *(h)*	human recombinant (HEK-293 cells)	(+)butaclamol	Grandy et al. (1989)
D_3 *(h)*	human recombinant (CHO cells)	(+)butaclamol	Mackenzie et al. (1994)
$GABA_A$	rat cerebral cortex	muscimol	Snodgrass (1978)

Assay	Origin	Reference Compound	Bibliography
$GABA_{B(1b)}$ *(h)*	human recombinant (HEK-293 cells)	CGP 54626	Green et al. (2000)
AMPA	rat cerebral cortex	L-glutamate	Murphy et al. (1987)
Kainate	rat cerebral cortex	kainic acid	Monaghan and Cotman (1982)
NMDA	rat cerebral cortex	CGS 19755	Sills et al. (1991)
Glycine (strychnine-insensitive)	rat cerebral cortex	glycine	Siegel et al. (1995)
H_1 *(h)*	human recombinant (HEK-293 cells)	pyrilamine	Smit et al. (1996)
H_2 *(h)*	human recombinant (CHO cells)	cimetidine	Leurs et al. (1994)
H_3 *(h)*	human recombinant (CHO cells)	(R)α-Me-histamine	Lovenberg et al. (1999)
MAO-A	rat cerebral cortex	clorgyline	Cesura et al. (1990)
M_1 *(h)*	human recombinant (CHO cells)	pirenzepine	Dorje et al. (1991)
M_2 *(h)*	human recombinant (CHO cells)	methoctramine	Dorje et al. (1991)
M_3 *(h)*	human recombinant (CHO cells)	4-DAMP	Peralta et al. (1987)
N (neuronal) (α-BGTX-insensitive) (α4β2)	rat cerebral cortex	nicotine	Pabreza et al. (1991)
N (muscle-type) *(h)*	TE671 cells	α-bungarotoxin	Lukas (1986)
δ_2 *(h)* (DOP)	human recombinant (CHO cells)	DPDPE	Simonin et al. (1994)
κ (KOP) (guinea-pig)	guinea-pig cerebellum	U 50488	Kinouchi and Pasternak (1991)
μ *(h)* (MOP) (agonist site)	human recombinant (HEK-293 cells)	DAMGO	Wang et al. (1994)
PPARγ *(h)*	human recombinant (*E. coli*)	rosiglitazone	Ferry et al. (2001)
$5\text{-}HT_{1A}$ *(h)*	human recombinant (HEK-293 cells)	8-OH-DPAT	Mulheron et al. (1994)
$5\text{-}HT_{1B}$	rat cerebral cortex	serotonin	Hoyer et al. (1985)
$5\text{-}HT_{2A}$ *(h)* (agonist site)	human recombinant (HEK-293 cells)	(±)DOI	Bryant et al. (1996)
$5\text{-}HT_{2B}$ *(h)* (agonist site)	human recombinant (CHO cells)	(±)DOI	Choi et al. (1994)

Assay	Origin	Reference Compound	Bibliography
5-HT_{2C} *(h)* (agonist site)	human recombinant (CHO cells)	(±)DOI	Bryant et al. (1996)
5-HT_3 *(h)*	human recombinant (CHO cells)	MDL 72222	Hope et al. (1996)
5-HT_{4e} *(h)*	human recombinant (CHO cells)	serotonin	Mialet et al. (2000)
5-HT_7 *(h)*	human recombinant (CHO cells)	serotonin	Shen et al. (1993)
Glucocorticoid *(h)* (GR)	IM-9 cells (cytosol)	dexamethasone	Clark et al. (1996)
V_{1a} *(h)*	human recombinant (CHO cells)	$[d(CH_2)_5{}^1,Tyr(Me)_2]$-AVP	Tahara et al. (1998)
Ca^{2+} channel (L, DHP site)	rat cerebral cortex	nitrendipine	Lee et al. (1984)
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)	rat cerebral cortex	diltiazem	Schoemaker and Langer (1985)
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	rat cerebral cortex	D 600	Reynolds et al. (1986)
Ca^{2+} channel (N)	rat cerebral cortex	ω-conotoxin GVIA	Wagner et al. (1988)
Na^+ channel (site 2)	rat cerebral cortex	veratridine	Brown (1986)
Cl^- channel	rat cerebral cortex	picrotoxinin	Lewin et al. (1989)
NE transporter *(h)*	human recombinant (CHO cells)	protriptyline	Pacholczyk et al. (1991)
DA transporter *(h)*	human recombinant (CHO cells)	BTCP	Pristupa et al. (1994)
GABA transporter	rat cerebral cortex	nipecotic acid	Shank et al. (1990)
Choline transporter *(h)* (CHT1)	human recombinant (CHO cells)	hemicholinium-3	Apparsundaram et al. (2000)
5-HT transporter *(h)*	human recombinant (CHO cells)	imipramine	Tatsumi et al. (1999)

2.1.2. Experimental Conditions

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
A_1 *(h)*	[^{3}H]DPCPX	1 nM	DPCPX (1 µM)	60 min./22°C	Scintillation counting
A_{2A} *(h)*	[^{3}H]CGS 21680	6 nM	NECA (10 µM)	120 min./22°C	Scintillation counting
α_1 (non-selective)	[^{3}H]prazosin	0.25 nM	prazosin (0.5 µM)	60 min./22°C	Scintillation counting
α_{2A} *(h)*	[^{3}H]RX 821002	1 nM	(-)epinephrine (100 µM)	60 min./22°C	Scintillation counting
α_{2B} *(h)*	[^{3}H]RX 821002	2.5 nM	(-)epinephrine (100 µM)	60 min./22°C	Scintillation counting
β_1 *(h)*	[^{3}H](-)CGP 12177	0.15 nM	alprenolol (50 µM)	60 min./22°C	Scintillation counting
β_2 *(h)*	[^{3}H](-)CGP 12177	0.15 nM	alprenolol (50 µM)	60 min./22°C	Scintillation counting
AT_1 *(h)*	[^{125}I][Sar^1,Ile^8]-AT II	0.05 nM	angiotensin II (10 µM)	120 min./37°C	Scintillation counting
BZD (central)	[^{3}H]flunitrazepam	0.4 nM	diazepam (3 µM)	60 min./4°C	Scintillation counting
CB_1 *(h)*	[^{3}H]CP 55940	0.5 nM	WIN 55212-2 (10 µM)	120 min./37°C	Scintillation counting
CB_2 *(h)*	[^{3}H]WIN 55212-2	0.8 nM	WIN 55212-2 (5 µM)	120 min./37°C	Scintillation counting
CCK_A *(h)* (CCK_1)	[^{125}I]CCK-8	0.08 nM	CCK-8 (1 µM)	60 min./22°C	Scintillation counting
CCK_B *(h)* (CCK_2)	[^{125}I]CCK-8	0.054 nM	CCK-8 (1 µM)	60 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
D_1 *(h)*	[^{3}H]SCH 23390	0.3 nM	SCH 23390 (1 µM)	60 min./22°C	Scintillation counting
D_{2S} *(h)*	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C	Scintillation counting
D_3 *(h)*	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C	Scintillation counting
$GABA_A$	[^{3}H]muscimol	5 nM	muscimol (10 µM)	10 min./4°C	Scintillation counting
$GABA_{B(1b)}$ *(h)*	[^{3}H]CGP 54626	2.5 nM	GABA (10 mM)	60 min./22°C	Scintillation counting
AMPA	[^{3}H]AMPA	8 nM	L-glutamate (1 mM)	60 min./4°C	Scintillation counting
Kainate	[^{3}H]kainic acid	5 nM	L-glutamate (1 mM)	60 min./4°C	Scintillation counting
NMDA	[^{3}H]CGP 39653	5 nM	L-glutamate (100 µM)	60 min./4°C	Scintillation counting
Glycine (strychnine-insensitive)	[^{3}H]MDL 105,519	0.5 nM	glycine (1 mM)	45 min./0°C	Scintillation counting
H_1 *(h)*	[^{3}H]pyrilamine	3 nM	pyrilamine (1 µM)	60 min./22°C	Scintillation counting
H_2 *(h)*	[^{125}I]APT	0.2 nM	tiotidine (100 µM)	120 min./22°C	Scintillation counting
H_3 *(h)*	[^{3}H]N$^{\alpha}$-Me-histamine	1 nM	(R)α-Me-histamine (1 µM)	60 min./22°C	Scintillation counting
MAO-A	[^{3}H]Ro 41-1049	10 nM	clorgyline (1 µM)	60 min/37°C	Scintillation counting
M_1 *(h)*	[^{3}H]pirenzepine	2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
M_2 *(h)*	[^{3}H]AF-DX 384	2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting
M_3 *(h)*	[^{3}H]4-DAMP	0.2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting
N (neuronal) (α-BGTX-insensitive) (α4β2)	[^{3}H]cytisine	1.5 nM	nicotine (10 µM)	75 min./4°C	Scintillation counting
N (muscle-type) *(h)*	[^{125}I]α-bungarotoxin	2.5 nM	α-bungarotoxin (5 µM)	120 min./22°C	Scintillation counting
δ_2 *(h)* (DOP)	[^{3}H]DADLE	0.5 nM	naltrexone (10 µM)	120 min./22°C	Scintillation counting
κ (KOP) (guinea-pig)	[^{3}H]U 69593	0.7 nM	naloxone (10 µM)	80 min./22°C	Scintillation counting
µ *(h)* (MOP) (agonist site)	[^{3}H]DAMGO	0.5 nM	naloxone (10 µM)	120 min./22°C	Scintillation counting
PPARγ *(h)*	[^{3}H]rosiglitazone	10 nM	rosiglitazone (10 µM)	120 min./4°C	Scintillation counting
5-HT_{1A} *(h)*	[^{3}H]8-OH-DPAT	0.3 nM	8-OH-DPAT (10 µM)	60 min./22°C	Scintillation counting
5-HT_{1B}	[^{125}I]CYP (+ 30 µM (-)propranolol)	0.1 nM	serotonin (10 µM)	120 min./37°C	Scintillation counting
5-HT_{2A} *(h)* (agonist site)	[^{125}I](±)DOI	0.2 nM	(±)DOI (1 µM)	60 min./22°C	Scintillation counting
5-HT_{2B} *(h)* (agonist site)	[^{125}I](±)DOI	0.2 nM	(±)DOI (1 µM)	15 min./37°C	Scintillation counting
5-HT_{2C} *(h)* (agonist site)	[^{125}I](±)DOI	0.2 nM	(±)DOI (10 µM)	15 min./37°C	Scintillation counting
5-HT_3 *(h)*	[^{3}H]BRL 43694	0.5 nM	MDL 72222 (10 µM)	120 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
5-HT_{4e} *(h)*	[^{3}H]GR 113808	0.3 nM	serotonin (100 µM)	60 min./37°C	Scintillation counting
5-HT_7 *(h)*	[^{3}H]LSD	4 nM	serotonin (10 µM)	120 min./22°C	Scintillation counting
Glucocorticoid *(h)* (GR)	[^{3}H]dexamethasone	1.5 nM	triamcinolone (10 µM)	6 h./4°C	Scintillation counting
V_{1a} *(h)*	[^{3}H]AVP	0.3 nM	AVP (1 µM)	60 min./22°C	Scintillation counting
Ca^{2+} channel (L, DHP site)	[^{3}H](+)PN 200-110	0.04 nM	nifedipine (1 µM)	90 min./22°C	Scintillation counting
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)	[^{3}H]diltiazem	5 nM	diltiazem (10 µM)	120 min./22°C	Scintillation counting
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	[^{3}H](-)D 888	3 nM	D 600 (10 µM)	120 min./22°C	Scintillation counting
Ca^{2+} channel (N)	[^{125}I]ω-conotoxin GVIA	0.001 nM	ω-conotoxin GVIA (10 nM)	30 min./22°C	Scintillation counting
Na^+ channel (site 2)	[^{3}H]batrachotoxinin	10 nM	veratridine (300 µM)	60 min./22°C	Scintillation counting
Cl^- channel	[^{35}S]TBPS	3 nM	picrotoxinin (20 µM)	120 min./22°C	Scintillation counting
NE transporter *(h)*	[^{3}H]nisoxetine	1 nM	desipramine (1 µM)	120 min./4°C	Scintillation counting
DA transporter *(h)*	[^{3}H]BTCP	4 nM	BTCP (10 µM)	120 min./4°C	Scintillation counting
GABA transporter	[^{3}H]GABA (+ 10 µM isoguvacine) (+ 10 µM baclofen)	10 nM	GABA (1 mM)	30 min./22°C	Scintillation counting
Choline transporter *(h)* (CHT1)	[^{3}H]hemicholinium-3	3 nM	hemicholinium-3 (10 µM)	60 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
5-HT transporter *(h)*	[^{3}H]imipramine	2 nM	imipramine (10 µM)	60 min./22°C	Scintillation counting

2.1.3. Analysis and Expression of Results

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand.

The results are expressed as a percent of control specific binding ((measured specific binding/control specific binding) x 100) and as a percent inhibition of control specific binding (100-((measured specific binding/control specific binding) x 100)) obtained in the presence of PF-04542563-00.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (*nH*) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/C_{50})^{nH})]$, where Y = specific binding, D = minimum specific binding, A = maximum specific binding, C = compound concentration, $C_{50} = IC_{50}$, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot ® 4.0 for Windows ® (© 1997 by SPSS Inc.).
The inhibition constants (K_i) were calculated using the Cheng Prùsoff equation ($K_i = IC_{50}/(1+(L/K_D))$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor).

2.2. *IN VITRO* PHARMACOLOGY: Enzyme Assays

2.2.1. General Procedures

Assay	Origin	Reference Compound	Bibliography
COX_2 *(h)*	human recombinant (Sf9 cells)	NS398	Glaser et al. (1995)
PDE3 *(h)*	human platelets	milrinone	Weishaar et al. (1986)
PDE4 *(h)*	U-937 cells	rolipram	Torphy et al. (1992)
ACE *(h)*	human recombinant (murine cells)	captopril	Hoorn and Roth (1993)
FLT-1 kinase *(h)* (VEGFR1)	human recombinant (Sf9 cells)	staurosporine	Itokawa et al. (2002)
p38α kinase *(h)*	human recombinant (*E. coli*)	SB202190	Frantz et al. (1998)
Acetylcholinesterase *(h)*	human recombinant (HEK-293 cells)	neostigmine	Ellman et al. (1961)
ATPase (Na^+/K^+)	porcine cerebral cortex	ouabain	Fiske and Subbarow (1925)

2.2.2. Experimental Conditions

Assay	Substrate/Stimulus/Tracer	Incubation	Reaction Product	Method of Detection
COX_2 *(h)*	arachidonic acid (2 µM)	5 min./22°C	PGE_2	EIA
PDE3 *(h)*	[^{3}H]cAMP + cAMP (0.1 µM)	60 min./22°C	[^{3}H]5'AMP	Scintillation counting
PDE4 *(h)*	[^{3}H]cAMP + cAMP (1 µM)	60 min./22°C	[^{3}H]5'AMP	Scintillation counting
ACE *(h)*	Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys (DNP)-OH (10 µM)	20 min./22°C	Mca-peptides	Fluorimetry
FLT-1 kinase *(h)* (VEGFR1)	ATP + biotinyl-βAβAβAAEEEEYFELVA KKK (0.5 µM)	20 min./22°C	phospho-biotinyl-βAβAβAAEEEEYFELVA KKK	HTRF
p38α kinase *(h)*	ATP + ATF-2 (0.1 µM)	30 min./22°C	phospho-ATF-2	HTRF

Assay	Substrate/Stimulus/Tracer	Incubation	Reaction Product	Method of Detection
Acetylcholinesterase *(h)*	AMTCh (50 µM)	30 min./37°C	thio-conjugate	Photometry
ATPase (Na^+/K^+)	ATP (2 mM)	60 min./37°C	Pi	Photometry

2.2.3. Analysis and Expression of Results

The results are expressed as a percent of control specific activity ((measured specific activity/control specific activity) x 100) and as a percent inhibition of control specific activity (100 – ((measured specific activity/control specific activity) x 100)) obtained in the presence of PF-04542563-00.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific activity) and Hill coefficients (*nH*) were determined by non-linear regression analysis of the inhibition curves generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/C_{50})^{nH})]$, where Y = specific activity, D = minimum specific activity, A = maximum specific activity, C = compound concentration, $C_{50} = IC_{50}$, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot ® 4.0 for Windows ® (© 1997 by SPSS Inc.).

3. COMPOUNDS

3.1. Test Compound

From: PFIZER Limited

CEREP I.D.	Compound I.D.	Reference Number	Batch Number	Submitted F.W.	Molecular Weight	Stock Solution	Intermediate Dilution
7570671-2	PF-04542563-00	7570671-002	PF-04542563-00-0001	243.82	235.33	1.E-02 M DMSO	1.E-04 M H2O 1.E-03 M H2O* [100x] DMSO**

F.W.: Formula Weight

*: For final test concentrations higher than 1.E-05 M.

**: For the human CB_1 assay.

3.2. Reference Compounds

In each experiment, the respective reference compound was tested concurrently with PF-04542563-00 in order to assess the assay suitability. It was tested at several concentrations (for IC_{50} value determination), and the data were compared with historical values determined at Cerep. The assay was rendered valid if the suitability criteria were met, in accordance with the corresponding Standard Operating Procedure.

4. RESULTS

4.1. *IN VITRO* PHARMACOLOGY: Binding Assays

The mean values for the effects of PF-04542563-00 are summarized in table 1 - 1.
The individual data obtained with PF-04542563-00 are reported in table 1 - 2.
The IC_{50} and K_i values for each reference compound are indicated in table 1 - 3. Each is within accepted limits of the historic average ± 0.5 log units.

The IC_{50} and K_i values determined for PF-04542563-00 are indicated in table 1 - 4.
The corresponding competition curves obtained with PF-04542563-00 are shown in figures 1 and 2.
The individual data obtained with PF-04542563-00 are reported in table 1 - 5.
The IC_{50} and K_i values for each reference compound are indicated in table 1 - 6. Each is within accepted limits of the historic average ± 0.5 log units.

Table 1 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
A_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	5
A_{2A} *(h)* 7570671-2	PF-04542563-00	1.0E-05	-3
α_1 (non-selective) 7570671-2	PF-04542563-00	1.0E-05	7
α_{2A} *(h)* 7570671-2	PF-04542563-00	1.0E-05	25
α_{2B} *(h)* 7570671-2	PF-04542563-00	1.0E-05	41
β_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	0
β_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	3
AT_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	-6
BZD (central) 7570671-2	PF-04542563-00	1.0E-05	8
CB_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	11
CB_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	-2
CCK_A *(h)* (CCK_1) 7570671-2	PF-04542563-00	1.0E-05	-11
CCK_B *(h)* (CCK_2) 7570671-2	PF-04542563-00	1.0E-05	4
D_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	-9
D_{2S} *(h)* 7570671-2	PF-04542563-00	1.0E-05	5
D_3 *(h)* 7570671-2	PF-04542563-00	1.0E-05	55
$GABA_A$ 7570671-2	PF-04542563-00	1.0E-05	-19
$GABA_{B(1b)}$ *(h)* 7570671-2	PF-04542563-00	1.0E-05	11
AMPA 7570671-2	PF-04542563-00	1.0E-05	-16
Kainate 7570671-2	PF-04542563-00	1.0E-05	2
NMDA 7570671-2	PF-04542563-00	1.0E-05	7

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
Glycine (strychnine-insensitive)			
7570671-2	PF-04542563-00	1.0E-05	-4
H_1 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	6
H_2 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	14
H_3 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	30
MAO-A			
7570671-2	PF-04542563-00	1.0E-05	18
M_1 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	31
M_2 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	25
M_3 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	**51**
N (neuronal) (α-BGTX-insensitive) (α4β2)			
7570671-2	PF-04542563-00	1.0E-05	11
N (muscle-type) *(h)*			
7570671-2	PF-04542563-00	1.0E-05	4
δ_2 *(h)* (DOP)			
7570671-2	PF-04542563-00	1.0E-05	-9
κ (KOP) (guinea-pig)			
7570671-2	PF-04542563-00	1.0E-05	11
μ *(h)* (MOP) (agonist site)			
7570671-2	PF-04542563-00	1.0E-05	18
PPARγ *(h)*			
7570671-2	PF-04542563-00	1.0E-05	8
5-HT_{1A} *(h)*			
7570671-2	PF-04542563-00	1.0E-05	-8
5-HT_{1B}			
7570671-2	PF-04542563-00	1.0E-05	0
5-HT_{2A} *(h)* (agonist site)			
7570671-2	PF-04542563-00	1.0E-05	12
5-HT_{2B} *(h)* (agonist site)			
7570671-2	PF-04542563-00	1.0E-05	10
5-HT_{2C} *(h)* (agonist site)			
7570671-2	PF-04542563-00	1.0E-05	-6
5-HT_3 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	2
5-HT_{4e} *(h)*			
7570671-2	PF-04542563-00	1.0E-05	1
5-HT_7 *(h)*			
7570671-2	PF-04542563-00	1.0E-05	8
Glucocorticoid *(h)* (GR)			
7570671-2	PF-04542563-00	1.0E-05	5
V_{1a} *(h)*			
7570671-2	PF-04542563-00	1.0E-05	7

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
Ca^{2+} channel (L, DHP site)			
7570671-2	PF-04542563-00	1.0E-05	12
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)			
7570671-2	PF-04542563-00	1.0E-05	6
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)			
7570671-2	PF-04542563-00	1.0E-05	1
Ca^{2+} channel (N)			
7570671-2	PF-04542563-00	1.0E-05	-2
Na^{+} channel (site 2)			
7570671-2	PF-04542563-00	1.0E-05	4
Cl^{-} channel			
7570671-2	PF-04542563-00	1.0E-05	3
NE transporter *(h)*			
7570671-2	PF-04542563-00	1.0E-05	2
DA transporter *(h)*			
7570671-2	PF-04542563-00	1.0E-05	8
GABA transporter			
7570671-2	PF-04542563-00	1.0E-05	2
Choline transporter *(h)* (CHT1)			
7570671-2	PF-04542563-00	1.0E-05	-5
5-HT transporter *(h)*			
7570671-2	PF-04542563-00	1.0E-05	-13

Table 1 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
A_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	91.7	98.8	**95.3**
A_{2A} *(h)* 7570671-2	PF-04542563-00	1.0E-05	105.3	101.2	**103.3**
α_1 (non-selective) 7570671-2	PF-04542563-00	1.0E-05	91.8	94.3	**93.0**
α_{2A} *(h)* 7570671-2	PF-04542563-00	1.0E-05	72.8	77.8	**75.3**
α_{2B} *(h)* 7570671-2	PF-04542563-00	1.0E-05	63.7	54.1	**58.9**
β_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	95.9	103.3	**99.6**
β_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	97.2	96.3	**96.8**
AT_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	104.3	107.5	**105.9**
BZD (central) 7570671-2	PF-04542563-00	1.0E-05	93.5	91.0	**92.3**
CB_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	88.5	90.2	**89.3**
CB_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	107.4	96.2	**101.8**
CCK_A *(h)* (CCK_1) 7570671-2	PF-04542563-00	1.0E-05	117.0	104.5	**110.8**
CCK_B *(h)* (CCK_2) 7570671-2	PF-04542563-00	1.0E-05	96.8	96.0	**96.4**
D_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	108.3	109.6	**109.0**
D_{2S} *(h)* 7570671-2	PF-04542563-00	1.0E-05	91.8	98.8	**95.3**
D_3 *(h)* 7570671-2	PF-04542563-00	1.0E-05	46.8	42.6	**44.7**
$GABA_A$ 7570671-2	PF-04542563-00	1.0E-05	121.5	115.6	**118.5**
$GABA_{B(1b)}$ *(h)* 7570671-2	PF-04542563-00	1.0E-05	81.4	96.1	**88.8**
AMPA 7570671-2	PF-04542563-00	1.0E-05	109.7	121.6	**115.6**
Kainate 7570671-2	PF-04542563-00	1.0E-05	99.7	96.0	**97.8**
NMDA 7570671-2	PF-04542563-00	1.0E-05	95.5	90.9	**93.2**

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
Glycine (strychnine-insensitive) 7570671-2	PF-04542563-00	1.0E-05	107.9	100.3	**104.1**
H_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	87.4	100.0	**93.7**
H_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	82.0	90.8	**86.4**
H_3 *(h)* 7570671-2	PF-04542563-00	1.0E-05	70.4	68.7	**69.6**
MAO-A 7570671-2	PF-04542563-00	1.0E-05	83.7	80.5	**82.1**
M_1 *(h)* 7570671-2	PF-04542563-00	1.0E-05	70.5	67.1	**68.8**
M_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	75.0	76.0	**75.5**
M_3 *(h)* 7570671-2	PF-04542563-00	1.0E-05	50.3	48.2	**49.2**
N (neuronal) (α-BGTX-insensitive) (α4β2) 7570671-2	PF-04542563-00	1.0E-05	92.2	86.7	**89.5**
N (muscle-type) *(h)* 7570671-2	PF-04542563-00	1.0E-05	96.5	94.8	**95.7**
δ_2 *(h)* (DOP) 7570671-2	PF-04542563-00	1.0E-05	106.8	110.5	**108.7**
κ (KOP) (guinea-pig) 7570671-2	PF-04542563-00	1.0E-05	91.8	85.3	**88.5**
μ *(h)* (MOP) (agonist site) 7570671-2	PF-04542563-00	1.0E-05	82.1	82.5	**82.3**
PPARγ *(h)* 7570671-2	PF-04542563-00	1.0E-05	94.1	89.1	**91.6**
5-HT_{1A} *(h)* 7570671-2	PF-04542563-00	1.0E-05	112.5	102.7	**107.6**
5-HT_{1B} 7570671-2	PF-04542563-00	1.0E-05	88.9	111.7	**100.3**
5-HT_{2A} *(h)* (agonist site) 7570671-2	PF-04542563-00	1.0E-05	78.5	98.3	**88.4**
5-HT_{2B} *(h)* (agonist site) 7570671-2	PF-04542563-00	1.0E-05	84.0	95.4	**89.7**
5-HT_{2C} *(h)* (agonist site) 7570671-2	PF-04542563-00	1.0E-05	113.4	99.2	**106.3**
5-HT_3 *(h)* 7570671-2	PF-04542563-00	1.0E-05	97.9	97.9	**97.9**
5-HT_{4e} *(h)* 7570671-2	PF-04542563-00	1.0E-05	100.5	97.8	**99.2**
5-HT_7 *(h)* 7570671-2	PF-04542563-00	1.0E-05	87.3	96.7	**92.0**
Glucocorticoid *(h)* (GR) 7570671-2	PF-04542563-00	1.0E-05	96.5	92.6	**94.5**
V_{1a} *(h)* 7570671-2	PF-04542563-00	1.0E-05	93.6	93.4	**93.5**

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
Ca^{2+} channel (L, DHP site)					
7570671-2	PF-04542563-00	1.0E-05	86.7	90.2	**88.4**
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)					
7570671-2	PF-04542563-00	1.0E-05	93.0	94.3	**93.6**
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)					
7570671-2	PF-04542563-00	1.0E-05	100.0	97.9	**99.0**
Ca^{2+} channel (N)					
7570671-2	PF-04542563-00	1.0E-05	103.8	100.4	**102.1**
Na^{+} channel (site 2)					
7570671-2	PF-04542563-00	1.0E-05	107.1	85.3	**96.2**
Cl^{-} channel					
7570671-2	PF-04542563-00	1.0E-05	93.6	99.7	**96.7**
NE transporter *(h)*					
7570671-2	PF-04542563-00	1.0E-05	89.8	105.4	**97.6**
DA transporter *(h)*					
7570671-2	PF-04542563-00	1.0E-05	89.7	94.6	**92.2**
GABA transporter					
7570671-2	PF-04542563-00	1.0E-05	90.7	105.1	**97.9**
Choline transporter *(h)* (CHT1)					
7570671-2	PF-04542563-00	1.0E-05	125.6	84.3	**105.0**
5-HT transporter *(h)*					
7570671-2	PF-04542563-00	1.0E-05	127.0	98.0	**112.5**

Table 1 - 3

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
A_1 *(h)* DPCPX	1.1E-08	7.0E-09	*1.0*
A_{2A} *(h)* NECA	4.5E-08	3.6E-08	*1.0*
α_1 (non-selective) prazosin	6.0E-10	1.6E-10	*1.1*
α_{2A} *(h)* yohimbine	5.7E-09	2.5E-09	*1.1*
α_{2B} *(h)* yohimbine	9.1E-09	6.1E-09	*1.1*
β_1 *(h)* atenolol	5.4E-07	3.9E-07	*1.1*
β_2 *(h)* ICI 118551	2.2E-09	9.0E-10	*1.3*
AT_1 *(h)* saralasin	8.8E-10	4.4E-10	*0.6*
BZD (central) diazepam	1.3E-08	1.1E-08	*1.4*
CB_1 *(h)* CP 55940	1.0E-09	8.8E-10	*1.2*
CB_2 *(h)* WIN 55212-2	3.0E-09	1.9E-09	*1.0*
CCK_A *(h)* (CCK_1) CCK-8	6.9E-10	5.2E-10	*1.3*
CCK_B *(h)* (CCK_2) CCK-8	7.9E-10	4.7E-10	*1.0*
D_1 *(h)* SCH 23390	9.9E-10	4.0E-10	*1.2*
D_{2S} *(h)* (+)butaclamol	7.7E-09	2.6E-09	*1.3*
D_3 *(h)* (+)butaclamol	4.9E-09	1.1E-09	*1.1*
$GABA_A$ muscimol	1.2E-08	8.3E-09	*1.9*
$GABA_{B(1b)}$ *(h)* CGP 54626	1.1E-08	4.8E-09	*1.1*
AMPA L-glutamate	3.2E-07	2.9E-07	*1.1*
Kainate kainic acid	1.0E-08	8.3E-09	*0.7*
NMDA CGS 19755	6.0E-07	4.9E-07	*1.1*
Glycine (strychnine-insensitive) glycine	3.2E-07	2.9E-07	*0.7*

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
H_1 *(h)* pyrilamine	3.6E-09	1.3E-09	*1.0*
H_2 *(h)* cimetidine	3.7E-07	3.5E-07	*1.0*
H_3 *(h)* (R)α-Me-histamine	1.2E-09	2.9E-10	*1.2*
MAO-A clorgyline	2.6E-09	1.5E-09	*1.4*
M_1 *(h)* pirenzepine	1.4E-08	1.2E-08	*0.9*
M_2 *(h)* methoctramine	4.1E-08	2.8E-08	*0.9*
M_3 *(h)* 4-DAMP	5.9E-10	4.2E-10	*1.2*
N (neuronal) (α-BGTX-insensitive) (α4β2) nicotine	8.9E-09	4.8E-09	*0.9*
N (muscle-type) *(h)* α-bungarotoxin	8.5E-09	6.7E-09	*1.2*
δ_2 *(h)* (DOP) DPDPE	3.2E-09	1.9E-09	*1.0*
κ (KOP) (guinea-pig) U 50488	5.6E-10	1.9E-10	*1.1*
μ *(h)* (MOP) (agonist site) DAMGO	6.8E-10	2.8E-10	*0.9*
PPARγ *(h)* rosiglitazone	3.7E-08	1.3E-08	*0.8*
5-HT_{1A} *(h)* 8-OH-DPAT	6.4E-10	4.0E-10	*1.0*
5-HT_{1B} serotonin	1.3E-08	8.3E-09	*0.7*
5-HT_{2A} *(h)* (agonist site) ()DOI	5.9E-10	3.6E-10	*0.7*
5-HT_{2B} *(h)* (agonist site) ()DOI	6.3E-09	6.1E-09	*0.7*
5-HT_{2C} *(h)* (agonist site) ()DOI	1.8E-09	1.4E-09	*0.6*
5-HT_3 *(h)* MDL 72222	8.6E-09	6.0E-09	*1.1*
5-HT_{4e} *(h)* serotonin	1.6E-07	5.3E-08	*0.6*
5-HT_7 *(h)* serotonin	8.7E-10	3.2E-10	*0.8*
Glucocorticoid *(h)* (GR) dexamethasone	3.9E-09	2.0E-09	*1.1*
V_{1a} *(h)* [d(CH_2)$_5$^{1},Tyr(Me)$_2$]-AVP	1.3E-09	8.0E-10	*1.0*
Ca^{2+} channel (L, DHP site) nitrendipine	6.8E-10	2.3E-10	*1.1*

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
Ca^{2+} channel (L, diltiazem site) (benzothiazepines) diltiazem	1.7E-08	1.5E-08	*1.5*
Ca^{2+} channel (L, verapamil site) (phenylalkylamines) D 600	6.5E-08	3.3E-08	*0.6*
Ca^{2+} channel (N) ω-conotoxin GVIA	1.1E-12	4.5E-13	*1.1*
Na^{+} channel (site 2) veratridine	4.7E-06	4.3E-06	*0.8*
Cl^{-} channel picrotoxinin	4.4E-07	3.6E-07	*0.8*
NE transporter *(h)* protriptyline	6.4E-09	4.8E-09	*1.0*
DA transporter *(h)* BTCP	8.4E-09	4.5E-09	*0.9*
GABA transporter nipecotic acid	9.9E-06	9.9E-06	*0.9*
Choline transporter *(h)* (CHT1) hemicholinium-3	1.3E-08	7.4E-09	*1.1*
5-HT transporter *(h)* imipramine	3.6E-09	1.7E-09	*1.2*

Table 1 - 4

IC_{50} Determination: Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	IC_{50} (M)	K_i (M)	n_H
D_3 *(h)*				
7570671-2	PF-04542563-00	8.6E-06	1.9E-06	*1.0*
M_3 *(h)*				
7570671-2	PF-04542563-00	1.2E-05	8.6E-06	*1.0*

COMPETITION CURVE OBTAINED WITH COMPOUND PF-04542563-00
AT THE HUMAN D3 RECEPTOR

IC50 = 8.6E-06 M
nH = 1.0

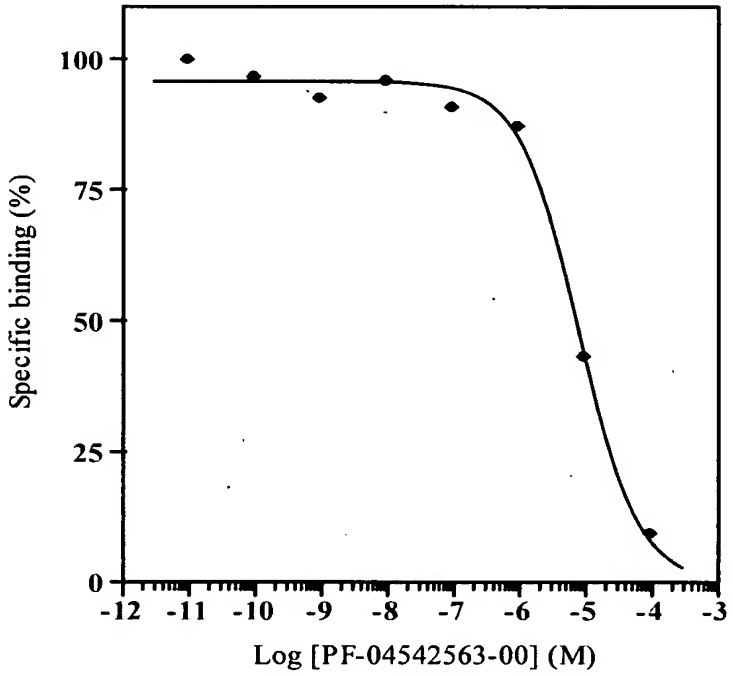


Figure 1

COMPETITION CURVE OBTAINED WITH COMPOUND PF-04542563-00
AT THE HUMAN M3 RECEPTOR

IC50 = 1.2E-05 M
nH = 1.0

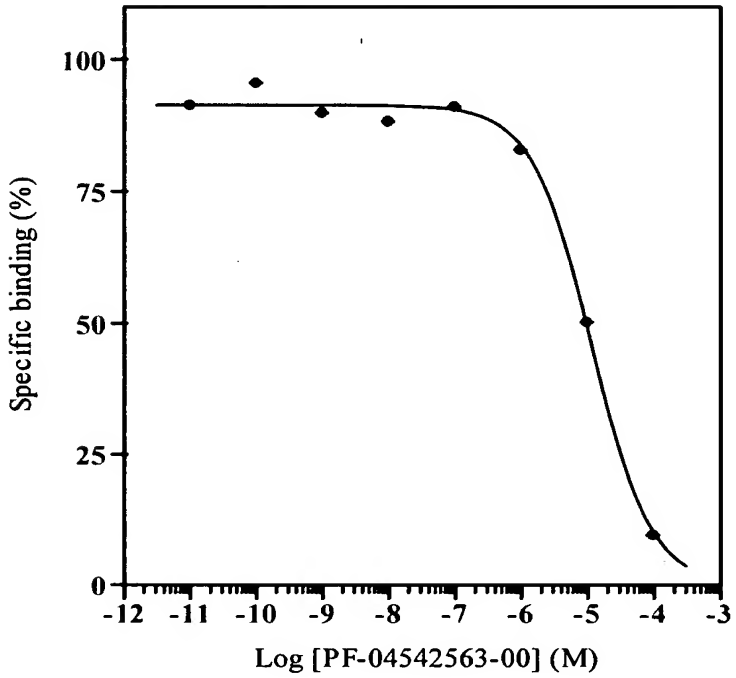


Figure 2

Table 1 - 5

IC_{50} Determination : Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	2nd	Mean
D_3 *(h)*					
7570671-2	PF-04542563-00	1.0E-11	110.0	89.9	**100.0**
7570671-2	PF-04542563-00	1.0E-10	105.7	87.5	**96.6**
7570671-2	PF-04542563-00	1.0E-09	95.3	89.8	**92.5**
7570671-2	PF-04542563-00	1.0E-08	97.7	94.0	**95.9**
7570671-2	PF-04542563-00	1.0E-07	90.4	91.2	**90.8**
7570671-2	PF-04542563-00	1.0E-06	89.0	85.1	**87.1**
7570671-2	PF-04542563-00	1.0E-05	44.4	42.2	**43.3**
7570671-2	PF-04542563-00	1.0E-04	8.7	10.2	**9.5**
M_3 *(h)*					
7570671-2	PF-04542563-00	1.0E-11	96.5	86.0	**91.3**
7570671-2	PF-04542563-00	1.0E-10	99.7	91.4	**95.6**
7570671-2	PF-04542563-00	1.0E-09	87.0	92.8	**89.9**
7570671-2	PF-04542563-00	1.0E-08	89.0	87.7	**88.3**
7570671-2	PF-04542563-00	1.0E-07	90.0	92.1	**91.0**
7570671-2	PF-04542563-00	1.0E-06	82.4	83.4	**82.9**
7570671-2	PF-04542563-00	1.0E-05	50.1	50.5	**50.3**
7570671-2	PF-04542563-00	1.0E-04	8.8	10.3	**9.6**

Table 1 - 6

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
D_3 *(h)* (+)butaclamol	5.6E-09	1.2E-09	*1.3*
M_3 *(h)* 4-DAMP	5.7E-10	4.1E-10	*1.3*

4.2. *IN VITRO* PHARMACOLOGY: Enzyme Assays

The mean values for the effects of PF-04542563-00 are summarized in table 2 - 1.
The individual data obtained with PF-04542563-00 are reported in table 2 - 2.

The IC_{50} value for each reference compound is indicated in table 2 - 3. Each is within accepted limits of the historic average ± 0.5 log units.

Table 2 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
COX_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	5
PDE3 *(h)* 7570671-2	PF-04542563-00	1.0E-05	3
PDE4 *(h)* 7570671-2	PF-04542563-00	1.0E-05	-4
ACE *(h)* 7570671-2	PF-04542563-00	1.0E-05	3
FLT-1 kinase *(h)* (VEGFR1) 7570671-2	PF-04542563-00	1.0E-05	1
p38α kinase *(h)* 7570671-2	PF-04542563-00	1.0E-05	7
Acetylcholinesterase *(h)* 7570671-2	PF-04542563-00	1.0E-05	-11
ATPase (Na^+/K^+) 7570671-2	PF-04542563-00	3.0E-05	6

Table 2 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	 2nd	 Mean
COX_2 *(h)* 7570671-2	PF-04542563-00	1.0E-05	97.0	92.4	**94.7**
PDE3 *(h)* 7570671-2	PF-04542563-00	1.0E-05	98.4	95.6	**97.0**
PDE4 *(h)* 7570671-2	PF-04542563-00	1.0E-05	101.9	105.6	**103.7**
ACE *(h)* 7570671-2	PF-04542563-00	1.0E-05	96.6	98.0	**97.3**
FLT-1 kinase *(h)* (VEGFR1) 7570671-2	PF-04542563-00	1.0E-05	95.6	102.9	**99.3**
p38α kinase *(h)* 7570671-2	PF-04542563-00	1.0E-05	93.0	93.0	**93.0**
Acetylcholinesterase *(h)* 7570671-2	PF-04542563-00	1.0E-05	111.6	110.4	**111.0**
ATPase (Na^+/K^+) 7570671-2	PF-04542563-00	3.0E-05	99.1	88.8	**94.0**

Table 2 - 3

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	n_H
COX_2 *(h)* NS398	1.0E-07	*1.9*
PDE3 *(h)* milrinone	1.6E-07	*0.9*
PDE4 *(h)* rolipram	3.0E-07	*0.7*
ACE *(h)* captopril	2.0E-09	*0.8*
FLT-1 kinase *(h)* (VEGFR1) staurosporine	8.9E-09	*1.6*
p38α kinase *(h)* SB202190	2.1E-08	*0.9*
Acetylcholinesterase *(h)* neostigmine	3.9E-08	*1.0*
ATPase (Na^+/K^+) ouabain	9.0E-07	*1.1*

5. HELP TO INTERPRET YOUR RESULTS IN *IN VITRO* PHARMACOLOGY

. Results showing an inhibition (or stimulation for assays run in basal conditions) higher than 50% are considered to represent significant effects of the test compounds. 50% is the most common cut-off value for further investigation (determination of IC_{50} or EC_{50} values from concentration-response curves).

. Results showing an inhibition (or stimulation) between 20% and 50% are indicative of weak to moderate effects (in some assays, they may be confirmed by further testing as they are within a range where more inter-experimental variability can occur).

. Results showing an inhibition (or stimulation) lower than 20% are not considered significant and mostly attributable to variability of the signal around the control level.

. Low to moderate negative values have no real meaning and are attributable to variability of the signal around the control level. High negative values (≥ 50%) that are sometimes obtained with high concentrations of test compounds are generally attributable to non-specific effects of the test compounds in the assays, apart from a few exceptions.

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7. STORAGE AND RETENTION OF RECORDS

All documents generated during the performance of the study (raw data, various recordings such as QA audit reports, an original of the study report, study plan...) will be stored for a 10-year period in Cerep's archive rooms after achievement of the study. Only Cerep's authorized employees shall have access to the archives.

The original final report provided to the sponsor will be kept by the sponsor under its sole responsibility.

8. QUALITY ASSURANCE STATEMENT

The following audit was performed on this study:

	CALENDAR
Audit of the Final Report	April 03, 2007

Audit report of the study report was transmitted to the Study Director for approval.

I certify that results presented in this report were generated using the materials and methods mentioned and that these results accurately reflect the Raw Data.

April 03, 2007

Nadine Pasquier

Quality Unit

Appendix 3

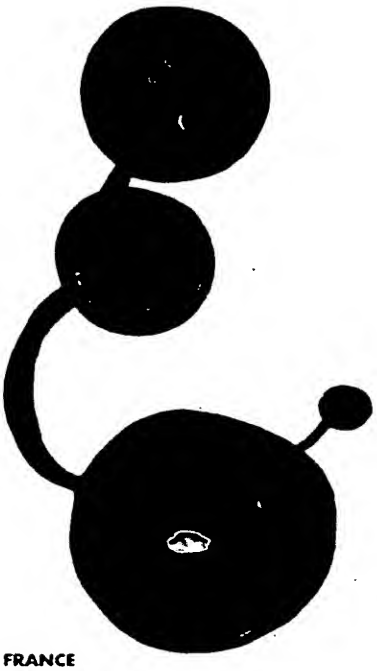
GLP Study report for binding assay 1 for Merck Example 3 enantiomer 2, completed at CEREP Biosciences

In this report, enantiomer 2 of Merck Example 3 is referred to by the reference number PF-4542565.

Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

Cerep

STUDY NUMBER 7570671a
FINAL REPORT



In Vitro Pharmacology: Pfizer Tier 0 Profile
- Study of PF-04542565-00 -

Annie OTTO-BRUC, Ph.D.

I FRANCE
Le Bois l'Evêque
86600 CELLE l'EVESCAULT
Tel. +33 (0)5 49 89 30 00
Fax +33 (0)5 49 43 21 70

(Headquarters)
155 boulevard Haussmann
75008 PARIS
Tel. +33 (0)1 45 64 44 60
Fax +33 (0)1 45 64 44 64

I USA
15318 N.E. 95th Street
REDMOND, WA 98052
Tel. : 1 425 895 8666
Fax : 1 425 895 8668

I www.cerep.com

I customer_support@cerep.fr

Cerep

Le Bois l'Evêque
B.P.1
86 600 Celle l'Evescault
France

www.cerep.com
Tel: 33 (0)5.49.89.30.00
Fax: 33 (0)5.49.43.21.70

Ref.: Final Report 7570671a/MN

STUDY NUMBER 7570671a

In Vitro Pharmacology: Pfizer Tier 0 Profile
- Study of PF-04542565-00 -

Study Sponsor:	PFIZER Limited
Attention:	Dr. Gareth WALDRON Senior Principal Scientist
Address:	Global Safety Pharmacology Ramsgate Road IPC 388 SANDWICH Kent CT13 9NJ UNITED KINGDOM
Study Director:	Annie OTTO-BRUC, Ph.D.
Testing Facility:	Cerep Le Bois l'Evêque - B.P. 1 - 86600 CELLE L'EVESCAULT, FRANCE
Study Period:	From March 06, 2007 to March 14, 2007
Report Version:	1
Report Date:	March 26, 2007

STUDY DIRECTOR

Annie OTTO-BRUC, Ph.D.
Principal Scientist, Pharmacology

March 26, 2007
Date

CONTENTS

1. PURPOSE OF THE STUDY

The purpose of this study was to investigate the effects of PF-04542565-00 in various *in vitro* receptor binding and enzyme assays.

2. MATERIALS AND METHODS

2.1. *IN VITRO* PHARMACOLOGY: Binding Assays

2.1.1. General Procedures

Assay	Origin	Reference Compound	Bibliography
A_1 *(h)*	human recombinant (CHO cells)	DPCPX	Townsend-Nicholson and Schofield (1994)
A_{2A} *(h)*	human recombinant (HEK-293 cells)	NECA	Luthin et al. (1995)
α_1 (non-selective)	rat cerebral cortex	prazosin	Greengrass and Bremner (1979)
α_{2A} *(h)*	human recombinant (CHO cells)	yohimbine	Langin et al. (1989)
α_{2B} *(h)*	human recombinant (CHO cells)	yohimbine	Devedjian et al. (1994)
β_1 *(h)*	human recombinant (HEK-293 cells)	atenolol	Levin et al. (2002)
β_2 *(h)*	human recombinant (Sf9 cells)	ICI 118551	Smith and Teitler (1999)
AT_1 *(h)*	human recombinant (HEK-293 cells)	saralasin	Le et al. (2005)
BZD (central)	rat cerebral cortex	diazepam	Speth et al. (1979)
CB_1 *(h)*	human recombinant (CHO cells)	CP 55940	Rinaldi-Carmona et al. (1996)
CB_2 *(h)*	human recombinant (CHO cells)	WIN 55212-2	Munro et al. (1993)
CCK_A *(h)* (CCK_1)	human recombinant (CHO cells)	CCK-8	Bignon et al. (1999)
CCK_B *(h)* (CCK_2)	human recombinant (CHO cells)	CCK-8	Lee et al. (1993)
D_1 *(h)*	human recombinant (CHO cells)	SCH 23390	Zhou et al. (1990)
D_{2S} *(h)*	human recombinant (HEK-293 cells)	(+)butaclamol	Grandy et al. (1989)
D_3 *(h)*	human recombinant (CHO cells)	(+)butaclamol	Mackenzie et al. (1994)
$GABA_A$	rat cerebral cortex	muscimol	Snodgrass (1978)

Assay	Origin	Reference Compound	Bibliography
$GABA_{B(1b)}$ *(h)*	human recombinant (HEK-293 cells)	CGP 54626	Green et al. (2000)
AMPA	rat cerebral cortex	L-glutamate	Murphy et al. (1987)
Kainate	rat cerebral cortex	kainic acid	Monaghan and Cotman (1982)
NMDA	rat cerebral cortex	CGS 19755	Sills et al. (1991)
Glycine (strychnine-insensitive)	rat cerebral cortex	glycine	Siegel et al. (1995)
H_1 *(h)*	human recombinant (HEK-293 cells)	pyrilamine	Smit et al. (1996)
H_2 *(h)*	human recombinant (CHO cells)	cimetidine	Leurs et al. (1994)
H_3 *(h)*	human recombinant (CHO cells)	(R)α-Me-histamine	Lovenberg et al. (1999)
MAO-A	rat cerebral cortex	clorgyline	Cesura et al. (1990)
M_1 *(h)*	human recombinant (CHO cells)	pirenzepine	Dorje et al. (1991)
M_2 *(h)*	human recombinant (CHO cells)	methoctramine	Dorje et al. (1991)
M_3 *(h)*	human recombinant (CHO cells)	4-DAMP	Peralta et al. (1987)
N (neuronal) (α-BGTX-insensitive) (α4β2)	rat cerebral cortex	nicotine	Pabreza et al. (1991)
N (muscle-type) *(h)*	TE671 cells	α-bungarotoxin	Lukas (1986)
δ_2 *(h)* (DOP)	human recombinant (CHO cells)	DPDPE	Simonin et al. (1994)
κ (KOP) (guinea-pig)	guinea-pig cerebellum	U 50488	Kinouchi and Pasternak (1991)
μ *(h)* (MOP) (agonist site)	human recombinant (HEK-293 cells)	DAMGO	Wang et al. (1994)
PPARγ *(h)*	human recombinant (*E. coli*)	rosiglitazone	Ferry et al. (2001)
$5\text{-}HT_{1A}$ *(h)*	human recombinant (HEK-293 cells)	8-OH-DPAT	Mulheron et al. (1994)
$5\text{-}HT_{1B}$	rat cerebral cortex	serotonin	Hoyer et al. (1985)
$5\text{-}HT_{2A}$ *(h)* (agonist site)	human recombinant (HEK-293 cells)	(±)DOI	Bryant et al. (1996)
$5\text{-}HT_{2B}$ *(h)* (agonist site)	human recombinant (CHO cells)	(±)DOI	Choi et al. (1994)

Assay	Origin	Reference Compound	Bibliography
5-HT_{2C} *(h)* (agonist site)	human recombinant (CHO cells)	(±)DOI	Bryant et al. (1996)
5-HT_3 *(h)*	human recombinant (CHO cells)	MDL 72222	Hope et al. (1996)
5-HT_{4e} *(h)*	human recombinant (CHO cells)	serotonin	Mialet et al. (2000)
5-HT_7 *(h)*	human recombinant (CHO cells)	serotonin	Shen et al. (1993)
Glucocorticoid *(h)* (GR)	IM-9 cells (cytosol)	dexamethasone	Clark et al. (1996)
V_{1a} *(h)*	human recombinant (CHO cells)	[d$(CH_2)_5^1$,Tyr$(Me)_2$]-AVP	Tahara et al. (1998)
Ca^{2+} channel (L, DHP site)	rat cerebral cortex	nitrendipine	Lee et al. (1984)
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)	rat cerebral cortex	diltiazem	Schoemaker and Langer (1985)
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	rat cerebral cortex	D 600	Reynolds et al. (1986)
Ca^{2+} channel (N)	rat cerebral cortex	ω-conotoxin GVIA	Wagner et al. (1988)
Na^+ channel (site 2)	rat cerebral cortex	veratridine	Brown (1986)
Cl^- channel	rat cerebral cortex	picrotoxinin	Lewin et al. (1989)
NE transporter *(h)*	human recombinant (CHO cells)	protriptyline	Pacholczyk et al. (1991)
DA transporter *(h)*	human recombinant (CHO cells)	BTCP	Pristupa et al. (1994)
GABA transporter	rat cerebral cortex	nipecotic acid	Shank et al. (1990)
Choline transporter *(h)* (CHT1)	human recombinant (CHO cells)	hemicholinium-3	Apparsundaram et al. (2000)
5-HT transporter *(h)*	human recombinant (CHO cells)	imipramine	Tatsumi et al. (1999)

2.1.2. Experimental Conditions

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
A_1 *(h)*	[^{3}H]DPCPX	1 nM	DPCPX (1 µM)	60 min./22°C	Scintillation counting
A_{2A} *(h)*	[^{3}H]CGS 21680	6 nM	NECA (10 µM)	120 min./22°C	Scintillation counting
α_1 (non-selective)	[^{3}H]prazosin	0.25 nM	prazosin (0.5 µM)	60 min./22°C	Scintillation counting
α_{2A} *(h)*	[^{3}H]RX 821002	1 nM	(-)epinephrine (100 µM)	60 min./22°C	Scintillation counting
α_{2B} *(h)*	[^{3}H]RX 821002	2.5 nM	(-)epinephrine (100 µM)	60 min./22°C	Scintillation counting
β_1 *(h)*	[^{3}H](-)CGP 12177	0.15 nM	alprenolol (50 µM)	60 min./22°C	Scintillation counting
β_2 *(h)*	[^{3}H](-)CGP 12177	0.15 nM	alprenolol (50 µM)	60 min./22°C	Scintillation counting
AT_1 *(h)*	[^{125}I][Sar^1,Ile^8]-AT II	0.05 nM	angiotensin II (10 µM)	120 min./37°C	Scintillation counting
BZD (central)	[^{3}H]flunitrazepam	0.4 nM	diazepam (3 µM)	60 min./4°C	Scintillation counting
CB_1 *(h)*	[^{3}H]CP 55940	0.5 nM	WIN 55212-2 (10 µM)	120 min./37°C	Scintillation counting
CB_2 *(h)*	[^{3}H]WIN 55212-2	0.8 nM	WIN 55212-2 (5 µM)	120 min./37°C	Scintillation counting
CCK_A *(h)* (CCK_1)	[^{125}I]CCK-8	0.08 nM	CCK-8 (1 µM)	60 min./22°C	Scintillation counting
CCK_B *(h)* (CCK_2)	[^{125}I]CCK-8	0.054 nM	CCK-8 (1 µM)	60 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
D_1 *(h)*	[^{3}H]SCH 23390	0.3 nM	SCH 23390 (1 µM)	60 min./22°C	Scintillation counting
D_{2S} *(h)*	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C	Scintillation counting
D_3 *(h)*	[^{3}H]spiperone	0.3 nM	(+)butaclamol (10 µM)	60 min./22°C	Scintillation counting
$GABA_A$	[^{3}H]muscimol	5 nM	muscimol (10 µM)	10 min./4°C	Scintillation counting
$GABA_{B(1b)}$ *(h)*	[^{3}H]CGP 54626	2.5 nM	GABA (10 mM)	60 min./22°C	Scintillation counting
AMPA	[^{3}H]AMPA	8 nM	L-glutamate (1 mM)	60 min./4°C	Scintillation counting
Kainate	[^{3}H]kainic acid	5 nM	L-glutamate (1 mM)	60 min./4°C	Scintillation counting
NMDA	[^{3}H]CGP 39653	5 nM	L-glutamate (100 µM)	60 min./4°C	Scintillation counting
Glycine (strychnine-insensitive)	[^{3}H]MDL 105,519	0.5 nM	glycine (1 mM)	45 min./0°C	Scintillation counting
H_1 *(h)*	[^{3}H]pyrilamine	3 nM	pyrilamine (1 µM)	60 min./22°C	Scintillation counting
H_2 *(h)*	[^{125}I]APT	0.2 nM	tiotidine (100 µM)	120 min./22°C	Scintillation counting
H_3 *(h)*	[^{3}H]N$^{\alpha}$-Me-histamine	1 nM	(R)α-Me-histamine (1 µM)	60 min./22°C	Scintillation counting
MAO-A	[^{3}H]Ro 41-1049	10 nM	clorgyline (1 µM)	60 min/37°C	Scintillation counting
M_1 *(h)*	[^{3}H]pirenzepine	2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
M_2 *(h)*	[^{3}H]AF-DX 384	2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting
M_3 *(h)*	[^{3}H]4-DAMP	0.2 nM	atropine (1 µM)	60 min./22°C	Scintillation counting
N (neuronal) (α-BGTX-insensitive) (α4β2)	[^{3}H]cytisine	1.5 nM	nicotine (10 µM)	75 min./4°C	Scintillation counting
N (muscle-type) *(h)*	[^{125}I]α-bungarotoxin	2.5 nM	α-bungarotoxin (5 µM)	120 min./22°C	Scintillation counting
δ_2 *(h)* (DOP)	[^{3}H]DADLE	0.5 nM	naltrexone (10 µM)	120 min./22°C	Scintillation counting
κ (KOP) (guinea-pig)	[^{3}H]U 69593	0.7 nM	naloxone (10 µM)	80 min./22°C	Scintillation counting
µ *(h)* (MOP) (agonist site)	[^{3}H]DAMGO	0.5 nM	naloxone (10 µM)	120 min./22°C	Scintillation counting
PPARγ *(h)*	[^{3}H]rosiglitazone	10 nM	rosiglitazone (10 µM)	120 min./4°C	Scintillation counting
5-HT_{1A} *(h)*	[^{3}H]8-OH-DPAT	0.3 nM	8-OH-DPAT (10 µM)	60 min./22°C	Scintillation counting
5-HT_{1B}	[^{125}I]CYP (+ 30 µM (-)propranolol)	0.1 nM	serotonin (10 µM)	120 min./37°C	Scintillation counting
5-HT_{2A} *(h)* (agonist site)	[^{125}I](±)DOI	0.2 nM	(±)DOI (1 µM)	60 min./22°C	Scintillation counting
5-HT_{2B} *(h)* (agonist site)	[^{125}I](±)DOI	0.2 nM	(±)DOI (1 µM)	15 min./37°C	Scintillation counting
5-HT_{2C} *(h)* (agonist site)	[^{125}I](±)DOI	0.2 nM	(±)DOI (10 µM)	15 min./37°C	Scintillation counting
5-HT_3 *(h)*	[^{3}H]BRL 43694	0.5 nM	MDL 72222 (10 µM)	120 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
5-HT_{4e} *(h)*	[^{3}H]GR 113808	0.3 nM	serotonin (100 µM)	60 min./37°C	Scintillation counting
5-HT_7 *(h)*	[^{3}H]LSD	4 nM	serotonin (10 µM)	120 min./22°C	Scintillation counting
Glucocorticoid *(h)* (GR)	[^{3}H]dexamethasone	1.5 nM	triamcinolone (10 µM)	6 h./4°C	Scintillation counting
V_{1a} *(h)*	[^{3}H]AVP	0.3 nM	AVP (1 µM)	60 min./22°C	Scintillation counting
Ca^{2+} channel (L, DHP site)	[^{3}H](+)PN 200-110	0.04 nM	nifedipine (1 µM)	90 min./22°C	Scintillation counting
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)	[^{3}H]diltiazem	5 nM	diltiazem (10 µM)	120 min./22°C	Scintillation counting
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	[^{3}H](-)D 888	3 nM	D 600 (10 µM)	120 min./22°C	Scintillation counting
Ca^{2+} channel (N)	[^{125}I]ω-conotoxin GVIA	0.001 nM	ω-conotoxin GVIA (10 nM)	30 min./22°C	Scintillation counting
Na^+ channel (site 2)	[^{3}H]batrachotoxinin	10 nM	veratridine (300 µM)	60 min./22°C	Scintillation counting
Cl^- channel	[^{35}S]TBPS	3 nM	picrotoxinin (20 µM)	120 min./22°C	Scintillation counting
NE transporter *(h)*	[^{3}H]nisoxetine	1 nM	desipramine (1 µM)	120 min./4°C	Scintillation counting
DA transporter *(h)*	[^{3}H]BTCP	4 nM	BTCP (10 µM)	120 min./4°C	Scintillation counting
GABA transporter	[^{3}H]GABA (+ 10 µM isoguvacine) (+ 10 µM baclofen)	10 nM	GABA (1 mM)	30 min./22°C	Scintillation counting
Choline transporter *(h)* (CHT1)	[^{3}H]hemicholinium-3	3 nM	hemicholinium-3 (10 µM)	60 min./22°C	Scintillation counting

Assay	Ligand	Conc.	Non Specific	Incubation	Method of Detection
5-HT transporter *(h)*	[^{3}H]imipramine	2 nM	imipramine (10 µM)	60 min./22°C	Scintillation counting

2.1.3. Analysis and Expression of Results

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand.

The results are expressed as a percent of control specific binding ((measured specific binding/control specific binding) x 100) and as a percent inhibition of control specific binding (100-((measured specific binding/control specific binding) x 100)) obtained in the presence of PF-04542565-00.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (*nH*) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/C_{50})^{nH})]$, where Y = specific binding, D = minimum specific binding, A = maximum specific binding, C = compound concentration, $C_{50} = IC_{50}$, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot ® 4.0 for Windows ® (© 1997 by SPSS Inc.).
The inhibition constants (K_i) were calculated using the Cheng Prusoff equation ($K_i = IC_{50}/(1+(L/K_D))$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor).

2.2. *IN VITRO* PHARMACOLOGY: Enzyme Assays

2.2.1. General Procedures

Assay	Origin	Reference Compound	Bibliography
COX_2 *(h)*	human recombinant (Sf9 cells)	NS398	Glaser et al. (1995)
PDE3 *(h)*	human platelets	milrinone	Weishaar et al. (1986)
PDE4 *(h)*	U-937 cells	rolipram	Torphy et al. (1992)
ACE *(h)*	human recombinant (murine cells)	captopril	Hoorn and Roth (1993)
FLT-1 kinase *(h)* (VEGFR1)	human recombinant (Sf9 cells)	staurosporine	Itokawa et al. (2002)
p38α kinase *(h)*	human recombinant (*E. coli*)	SB202190	Frantz et al. (1998)
Acetylcholinesterase *(h)*	human recombinant (HEK-293 cells)	neostigmine	Ellman et al. (1961)
ATPase (Na^+/K^+)	porcine cerebral cortex	ouabain	Fiske and Subbarow (1925)

2.2.2. Experimental Conditions

Assay	Substrate/Stimulus/Tracer	Incubation	Reaction Product	Method of Detection
COX_2 *(h)*	arachidonic acid (2 µM)	5 min./22°C	PGE_2	EIA
PDE3 *(h)*	[^{3}H]cAMP + cAMP (0.1 µM)	60 min./22°C	[^{3}H]5'AMP	Scintillation counting
PDE4 *(h)*	[^{3}H]cAMP + cAMP (1 µM)	60 min./22°C	[^{3}H]5'AMP	Scintillation counting
ACE *(h)*	Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys (DNP)-OH (10 µM)	20 min./22°C	Mca-peptides	Fluorimetry
FLT-1 kinase *(h)* (VEGFR1)	ATP + biotinyl-βAβAβAAEEEEYFELVAKKK (0.5 µM)	20 min./22°C	phospho-biotinyl-βAβAβAAEEEEYFELVAKKK	HTRF
p38α kinase *(h)*	ATP + ATF-2 (0.1 µM)	30 min./22°C	phospho-ATF-2	HTRF
Acetylcholinesterase *(h)*	AMTCh (50 µM)	30 min./37°C	thio-conjugate	Photometry

Assay	Substrate/Stimulus/Tracer	Incubation	Reaction Product	Method of Detection
ATPase (Na^+/K^+)	ATP (2 mM)	60 min./37°C	Pi	Photometry

2.2.3. Analysis and Expression of Results

The results are expressed as a percent of control specific activity ((measured specific activity/control specific activity) x 100) and as a percent inhibition of control specific activity (100 – ((measured specific activity/control specific activity) x 100)) obtained in the presence of PF-04542565-00.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific activity) and Hill coefficients (*nH*) were determined by non-linear regression analysis of the inhibition curves generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/C_{50})^{nH})]$, where Y = specific activity, D = minimum specific activity, A = maximum specific activity, C = compound concentration, $C_{50} = IC_{50}$, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot ® 4.0 for Windows ® (© 1997 by SPSS Inc.).

3. COMPOUNDS

3.1. Test Compound

From: PFIZER Limited

CEREP I.D.	Compound I.D.	Reference Number	Batch Number	Submitted F.W.	Molecular Weight	Stock Solution	Intermediate Dilution
7570671-1	PF-04542565-00	7570671-001	PF-04542595-00-0001	239.58	235.33	1.E-02 M DMSO	1.E-04 M H2O 3.E-04 M H2O* [100x] DMSO**

F.W.: Formula Weight

*: For ATPase (Na^+/K^+) assay.

**: For the human CB_1 assay.

3.2. Reference Compounds

In each experiment, the respective reference compound was tested concurrently with PF-04542565-00 in order to assess the assay suitability. It was tested at several concentrations (for IC_{50} value determination), and the data were compared with historical values determined at Cerep. The assay was rendered valid if the suitability criteria were met, in accordance with the corresponding Standard Operating Procedure.

4. RESULTS

4.1. *IN VITRO* PHARMACOLOGY: Binding Assays

The mean values for the effects of PF-04542565-00 are summarized in table 1 - 1.
The individual data obtained with PF-04542565-00 are reported in table 1 - 2.

The IC_{50} and K_i values for each reference compound are indicated in table 1 - 3. Each is within accepted limits of the historic average ± 0.5 log units.

Table 1 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
A_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	3
A_{2A} *(h)* 7570671-1	PF-04542565-00	1.0E-05	3
α_1 (non-selective) 7570671-1	PF-04542565-00	1.0E-05	2
α_{2A} *(h)* 7570671-1	PF-04542565-00	1.0E-05	0
α_{2B} *(h)* 7570671-1	PF-04542565-00	1.0E-05	18
β_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	-2
β_2 *(h)* 7570671-1	PF-04542565-00	1.0E-05	-1
AT_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	-6
BZD (central) 7570671-1	PF-04542565-00	1.0E-05	7
CB_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	3
CB_2 *(h)* 7570671-1	PF-04542565-00	1.0E-05	1
CCK_A *(h)* (CCK_1) 7570671-1	PF-04542565-00	1.0E-05	-12
CCK_B *(h)* (CCK_2) 7570671-1	PF-04542565-00	1.0E-05	5
D_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	-4
D_{2S} *(h)* 7570671-1	PF-04542565-00	1.0E-05	5
D_3 *(h)* 7570671-1	PF-04542565-00	1.0E-05	8
$GABA_A$ 7570671-1	PF-04542565-00	1.0E-05	2
$GABA_{B(1b)}$ *(h)* 7570671-1	PF-04542565-00	1.0E-05	6
AMPA 7570671-1	PF-04542565-00	1.0E-05	-19
Kainate 7570671-1	PF-04542565-00	1.0E-05	6
NMDA 7570671-1	PF-04542565-00	1.0E-05	9

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
Glycine (strychnine-insensitive)			
7570671-1	PF-04542565-00	1.0E-05	2
H_1 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	3
H_2 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	5
H_3 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	6
MAO-A			
7570671-1	PF-04542565-00	1.0E-05	-2
M_1 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	33
M_2 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	21
M_3 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	31
N (neuronal) (α-BGTX-insensitive) ($\alpha 4\beta 2$)			
7570671-1	PF-04542565-00	1.0E-05	17
N (muscle-type) *(h)*			
7570671-1	PF-04542565-00	1.0E-05	4
δ_2 *(h)* (DOP)			
7570671-1	PF-04542565-00	1.0E-05	3
κ (KOP) (guinea-pig)			
7570671-1	PF-04542565-00	1.0E-05	12
μ *(h)* (MOP) (agonist site)			
7570671-1	PF-04542565-00	1.0E-05	28
PPARγ *(h)*			
7570671-1	PF-04542565-00	1.0E-05	3
5-HT_{1A} *(h)*			
7570671-1	PF-04542565-00	1.0E-05	1
5-HT_{1B}			
7570671-1	PF-04542565-00	1.0E-05	-3
5-HT_{2A} *(h)* (agonist site)			
7570671-1	PF-04542565-00	1.0E-05	10
5-HT_{2B} *(h)* (agonist site)			
7570671-1	PF-04542565-00	1.0E-05	5
5-HT_{2C} *(h)* (agonist site)			
7570671-1	PF-04542565-00	1.0E-05	-9
5-HT_3 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	1
5-HT_{4e} *(h)*			
7570671-1	PF-04542565-00	1.0E-05	-2
5-HT_7 *(h)*			
7570671-1	PF-04542565-00	1.0E-05	0
Glucocorticoid *(h)* (GR)			
7570671-1	PF-04542565-00	1.0E-05	4
V_{1a} *(h)*			
7570671-1	PF-04542565-00	1.0E-05	7

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
Ca^{2+} channel (L, DHP site)			
7570671-1	PF-04542565-00	1.0E-05	3
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)			
7570671-1	PF-04542565-00	1.0E-05	10
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)			
7570671-1	PF-04542565-00	1.0E-05	-1
Ca^{2+} channel (N)			
7570671-1	PF-04542565-00	1.0E-05	1
Na^{+} channel (site 2)			
7570671-1	PF-04542565-00	1.0E-05	18
Cl^{-} channel			
7570671-1	PF-04542565-00	1.0E-05	-4
NE transporter *(h)*			
7570671-1	PF-04542565-00	1.0E-05	-5
DA transporter *(h)*			
7570671-1	PF-04542565-00	1.0E-05	7
GABA transporter			
7570671-1	PF-04542565-00	1.0E-05	6
Choline transporter *(h)* (CHT1)			
7570671-1	PF-04542565-00	1.0E-05	14
5-HT transporter *(h)*			
7570671-1	PF-04542565-00	1.0E-05	2

Table 1 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
A_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	95.0	98.5	**96.7**
A_{2A} *(h)* 7570671-1	PF-04542565-00	1.0E-05	99.4	94.7	**97.0**
α_1 (non-selective) 7570671-1	PF-04542565-00	1.0E-05	99.7	95.8	**97.8**
α_{2A} *(h)* 7570671-1	PF-04542565-00	1.0E-05	102.3	97.3	**99.8**
α_{2B} *(h)* 7570671-1	PF-04542565-00	1.0E-05	82.0	81.1	**81.6**
β_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	100.6	104.1	**102.3**
β_2 *(h)* 7570671-1	PF-04542565-00	1.0E-05	103.2	98.0	**100.6**
AT_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	103.2	107.9	**105.6**
BZD (central) 7570671-1	PF-04542565-00	1.0E-05	94.4	91.1	**92.8**
CB_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	93.6	99.8	**96.7**
CB_2 *(h)* 7570671-1	PF-04542565-00	1.0E-05	104.4	94.2	**99.3**
CCK_A *(h)* (CCK_1) 7570671-1	PF-04542565-00	1.0E-05	117.6	105.8	**111.7**
CCK_B *(h)* (CCK_2) 7570671-1	PF-04542565-00	1.0E-05	98.8	92.0	**95.4**
D_1 *(h)* 7570671-1	PF-04542565-00	1.0E-05	107.0	101.3	**104.1**
D_{2S} *(h)* 7570671-1	PF-04542565-00	1.0E-05	102.9	86.9	**94.9**
D_3 *(h)* 7570671-1	PF-04542565-00	1.0E-05	98.7	85.8	**92.3**
$GABA_A$ 7570671-1	PF-04542565-00	1.0E-05	104.5	90.7	**97.6**
$GABA_{B(1b)}$ *(h)* 7570671-1	PF-04542565-00	1.0E-05	83.9	105.1	**94.5**
AMPA 7570671-1	PF-04542565-00	1.0E-05	110.8	128.1	**119.4**
Kainate 7570671-1	PF-04542565-00	1.0E-05	92.3	96.0	**94.1**
NMDA 7570671-1	PF-04542565-00	1.0E-05	90.6	90.5	**90.6**

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	2nd	Mean
Glycine (strychnine-insensitive)					
7570671-1	PF-04542565-00	1.0E-05	98.4	96.7	**97.5**
H_1 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	100.5	94.1	**97.3**
H_2 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	89.5	100.6	**95.1**
H_3 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	90.4	97.6	**94.0**
MAO-A					
7570671-1	PF-04542565-00	1.0E-05	107.5	95.6	**101.6**
M_1 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	68.3	65.8	**67.0**
M_2 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	80.6	77.0	**78.8**
M_3 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	68.4	68.8	**68.6**
N (neuronal) (α-BGTX-insensitive) (α4β2)					
7570671-1	PF-04542565-00	1.0E-05	85.0	81.4	**83.2**
N (muscle-type) *(h)*					
7570671-1	PF-04542565-00	1.0E-05	98.5	93.5	**96.0**
δ_2 *(h)* (DOP)					
7570671-1	PF-04542565-00	1.0E-05	101.4	92.6	**97.0**
κ (KOP) (guinea-pig)					
7570671-1	PF-04542565-00	1.0E-05	83.6	92.9	**88.3**
μ *(h)* (MOP) (agonist site)					
7570671-1	PF-04542565-00	1.0E-05	74.1	70.1	**72.1**
PPARγ *(h)*					
7570671-1	PF-04542565-00	1.0E-05	94.0	99.2	**96.6**
5-HT_{1A} *(h)*					
7570671-1	PF-04542565-00	1.0E-05	104.4	94.2	**99.3**
5-HT_{1B}					
7570671-1	PF-04542565-00	1.0E-05	93.7	113.2	**103.4**
5-HT_{2A} *(h)* (agonist site)					
7570671-1	PF-04542565-00	1.0E-05	86.2	92.9	**89.5**
5-HT_{2B} *(h)* (agonist site)					
7570671-1	PF-04542565-00	1.0E-05	89.0	100.6	**94.8**
5-HT_{2C} *(h)* (agonist site)					
7570671-1	PF-04542565-00	1.0E-05	106.1	112.6	**109.4**
5-HT_3 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	94.2	103.5	**98.8**
5-HT_{4e} *(h)*					
7570671-1	PF-04542565-00	1.0E-05	102.8	100.3	**101.6**
5-HT_7 *(h)*					
7570671-1	PF-04542565-00	1.0E-05	104.4	95.5	**100.0**
Glucocorticoid *(h)* (GR)					
7570671-1	PF-04542565-00	1.0E-05	99.2	92.9	**96.1**
V_{1a} *(h)*					
7570671-1	PF-04542565-00	1.0E-05	93.6	92.0	**92.8**

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	 2nd	 Mean
Ca^{2+} channel (L, DHP site)					
7570671-1	PF-04542565-00	1.0E-05	95.9	97.4	**96.7**
Ca^{2+} channel (L, diltiazem site) (benzothiazepines)					
7570671-1	PF-04542565-00	1.0E-05	88.5	91.8	**90.2**
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)					
7570671-1	PF-04542565-00	1.0E-05	102.4	99.5	**100.9**
Ca^{2+} channel (N)					
7570671-1	PF-04542565-00	1.0E-05	100.4	97.7	**99.1**
Na^{+} channel (site 2)					
7570671-1	PF-04542565-00	1.0E-05	79.0	84.5	**81.7**
Cl^{-} channel					
7570671-1	PF-04542565-00	1.0E-05	103.5	104.8	**104.1**
NE transporter *(h)*					
7570671-1	PF-04542565-00	1.0E-05	106.4	102.8	**104.6**
DA transporter *(h)*					
7570671-1	PF-04542565-00	1.0E-05	99.7	87.1	**93.4**
GABA transporter					
7570671-1	PF-04542565-00	1.0E-05	103.3	84.9	**94.1**
Choline transporter *(h)* (CHT1)					
7570671-1	PF-04542565-00	1.0E-05	85.9	85.3	**85.6**
5-HT transporter *(h)*					
7570671-1	PF-04542565-00	1.0E-05	93.9	101.6	**97.8**

Table 1 - 3

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
A_1 *(h)* DPCPX	1.1E-08	7.0E-09	*1.0*
A_{2A} *(h)* NECA	4.5E-08	3.6E-08	*1.0*
α_1 (non-selective) prazosin	6.0E-10	1.6E-10	*1.1*
α_{2A} *(h)* yohimbine	5.7E-09	2.5E-09	*1.1*
α_{2B} *(h)* yohimbine	9.1E-09	6.1E-09	*1.1*
β_1 *(h)* atenolol	5.4E-07	3.9E-07	*1.1*
β_2 *(h)* ICI 118551	2.2E-09	9.0E-10	*1.3*
AT_1 *(h)* saralasin	8.8E-10	4.4E-10	*0.6*
BZD (central) diazepam	1.3E-08	1.1E-08	*1.4*
CB_1 *(h)* CP 55940	1.0E-09	8.8E-10	*1.2*
CB_2 *(h)* WIN 55212-2	3.0E-09	1.9E-09	*1.0*
CCK_A *(h)* (CCK_1) CCK-8	6.9E-10	5.2E-10	*1.3*
CCK_B *(h)* (CCK_2) CCK-8	7.9E-10	4.7E-10	*1.0*
D_1 *(h)* SCH 23390	9.9E-10	4.0E-10	*1.2*
D_{2S} *(h)* (+)butaclamol	7.7E-09	2.6E-09	*1.3*
D_3 *(h)* (+)butaclamol	4.9E-09	1.1E-09	*1.1*
$GABA_A$ muscimol	1.2E-08	8.3E-09	*1.9*
$GABA_{B(1b)}$ *(h)* CGP 54626	1.1E-08	4.8E-09	*1.1*
AMPA L-glutamate	3.2E-07	2.9E-07	*1.1*
Kainate kainic acid	1.0E-08	8.3E-09	*0.7*
NMDA CGS 19755	6.0E-07	4.9E-07	*1.1*
Glycine (strychnine-insensitive) glycine	3.2E-07	2.9E-07	*0.7*

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
H_1 *(h)* pyrilamine	3.6E-09	1.3E-09	*1.0*
H_2 *(h)* cimetidine	3.7E-07	3.5E-07	*1.0*
H_3 *(h)* (R)α-Me-histamine	1.2E-09	2.9E-10	*1.2*
MAO-A clorgyline	2.6E-09	1.5E-09	*1.4*
M_1 *(h)* pirenzepine	1.4E-08	1.2E-08	*0.9*
M_2 *(h)* methoctramine	4.1E-08	2.8E-08	*0.9*
M_3 *(h)* 4-DAMP	5.9E-10	4.2E-10	*1.2*
N (neuronal) (α-BGTX-insensitive) (α4β2) nicotine	8.9E-09	4.8E-09	*0.9*
N (muscle-type) *(h)* α-bungarotoxin	8.5E-09	6.7E-09	*1.2*
δ_2 *(h)* (DOP) DPDPE	3.2E-09	1.9E-09	*1.0*
κ (KOP) (guinea-pig) U 50488	5.6E-10	1.9E-10	*1.1*
μ *(h)* (MOP) (agonist site) DAMGO	6.8E-10	2.8E-10	*0.9*
PPARγ *(h)* rosiglitazone	3.7E-08	1.3E-08	*0.8*
5-HT_{1A} *(h)* 8-OH-DPAT	6.4E-10	4.0E-10	*1.0*
5-HT_{1B} serotonin	1.3E-08	8.3E-09	*0.7*
5-HT_{2A} *(h)* (agonist site) ()DOI	5.9E-10	3.6E-10	*0.7*
5-HT_{2B} *(h)* (agonist site) ()DOI	6.3E-09	6.1E-09	*0.7*
5-HT_{2C} *(h)* (agonist site) ()DOI	1.8E-09	1.4E-09	*0.6*
5-HT_3 *(h)* MDL 72222	8.6E-09	6.0E-09	*1.1*
5-HT_{4e} *(h)* serotonin	1.6E-07	5.3E-08	*0.6*
5-HT_7 *(h)* serotonin	8.7E-10	3.2E-10	*0.8*
Glucocorticoid *(h)* (GR) dexamethasone	3.9E-09	2.0E-09	*1.1*
V_{1a} *(h)* [$d(CH_2)_5^1$,Tyr$(Me)_2$]-AVP	1.3E-09	8.0E-10	*1.0*
Ca^{2+} channel (L, DHP site) nitrendipine	6.8E-10	2.3E-10	*1.1*

Assay Reference Compound	IC_{50} (M)	K_i (M)	n_H
Ca^{2+} channel (L, diltiazem site) (benzothiazepines) diltiazem	1.7E-08	1.5E-08	*1.5*
Ca^{2+} channel (L, verapamil site) (phenylalkylamines) D 600	6.5E-08	3.3E-08	*0.6*
Ca^{2+} channel (N) ω-conotoxin GVIA	1.1E-12	4.5E-13	*1.1*
Na^{+} channel (site 2) veratridine	4.7E-06	4.3E-06	*0.8*
Cl^{-} channel picrotoxinin	4.4E-07	3.6E-07	*0.8*
NE transporter *(h)* protriptyline	6.4E-09	4.8E-09	*1.0*
DA transporter *(h)* BTCP	8.4E-09	4.5E-09	*0.9*
GABA transporter nipecotic acid	9.9E-06	9.9E-06	*0.9*
Choline transporter *(h)* (CHT1) hemicholinium-3	1.3E-08	7.4E-09	*1.1*
5-HT transporter *(h)* imipramine	3.6E-09	1.7E-09	*1.2*

4.2. *IN VITRO* PHARMACOLOGY: Enzyme Assays

The mean values for the effects of PF-04542565-00 are summarized in table 2 - 1.
The individual data obtained with PF-04542565-00 are reported in table 2 - 2.

The IC_{50} value for each reference compound is indicated in table 2 - 3. Each is within accepted limits of the historic average ± 0.5 log units.

Table 2 - 1

Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
COX_2 *(h)* 7570671-1	PF-04542565-00	1.0E-05	4
PDE3 *(h)* 7570671-1	PF-04542565-00	1.0E-05	2
PDE4 *(h)* 7570671-1	PF-04542565-00	1.0E-05	-1
ACE *(h)* 7570671-1	PF-04542565-00	1.0E-05	3
FLT-1 kinase *(h)* (VEGFR1) 7570671-1	PF-04542565-00	1.0E-05	-1
p38α kinase *(h)* 7570671-1	PF-04542565-00	1.0E-05	2
Acetylcholinesterase *(h)* 7570671-1	PF-04542565-00	1.0E-05	-31
ATPase (Na^+/K^+) 7570671-1	PF-04542565-00	3.0E-05	3

Table 2 - 2

Individual Data

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Values 1st	 2nd	 Mean
COX_2 *(h)* 7570671-1	PF-04542565-00	1.0E-05	100.2	91.7	**96.0**
PDE3 *(h)* 7570671-1	PF-04542565-00	1.0E-05	98.1	97.4	**97.7**
PDE4 *(h)* 7570671-1	PF-04542565-00	1.0E-05	101.4	101.5	**101.5**
ACE *(h)* 7570671-1	PF-04542565-00	1.0E-05	98.6	94.7	**96.6**
FLT-1 kinase *(h)* (VEGFR1) 7570671-1	PF-04542565-00	1.0E-05	102.3	100.2	**101.3**
p38α kinase *(h)* 7570671-1	PF-04542565-00	1.0E-05	101.4	94.7	**98.0**
Acetylcholinesterase *(h)* 7570671-1	PF-04542565-00	1.0E-05	133.9	128.9	**131.4**
ATPase (Na^+/K^+) 7570671-1	PF-04542565-00	3.0E-05	93.1	100.7	**96.9**

Table 2 - 3

Reference Compound Data

Assay Reference Compound	IC_{50} (M)	n_H
COX_2 *(h)* NS398	1.0E-07	*1.9*
PDE3 *(h)* milrinone	1.6E-07	*0.9*
PDE4 *(h)* rolipram	3.0E-07	*0.7*
ACE *(h)* captopril	2.0E-09	*0.8*
FLT-1 kinase *(h)* (VEGFR1) staurosporine	8.9E-09	*1.6*
p38α kinase *(h)* SB202190	2.1E-08	*0.9*
Acetylcholinesterase *(h)* neostigmine	3.9E-08	*1.0*
ATPase (Na^+/K^+) ouabain	9.0E-07	*1.1*

5. HELP TO INTERPRET YOUR RESULTS IN *IN VITRO* PHARMACOLOGY

. Results showing an inhibition (or stimulation for assays run in basal conditions) higher than 50% are considered to represent significant effects of the test compounds. 50% is the most common cut-off value for further investigation (determination of IC_{50} or EC_{50} values from concentration-response curves).

. Results showing an inhibition (or stimulation) between 20% and 50% are indicative of weak to moderate effects (in some assays, they may be confirmed by further testing as they are within a range where more inter-experimental variability can occur).

. Results showing an inhibition (or stimulation) lower than 20% are not considered significant and mostly attributable to variability of the signal around the control level.

. Low to moderate negative values have no real meaning and are attributable to variability of the signal around the control level. High negative values (≥ 50%) that are sometimes obtained with high concentrations of test compounds are generally attributable to non-specific effects of the test compounds in the assays, apart from a few exceptions.

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7. STORAGE AND RETENTION OF RECORDS

All documents generated during the performance of the study (raw data, various recordings such as QA audit reports, an original of the study report, study plan...) will be stored for a 10-year period in Cerep's archive rooms after achievement of the study. Only Cerep's authorized employees shall have access to the archives.

The original final report provided to the sponsor will be kept by the sponsor under its sole responsibility.

8. QUALITY ASSURANCE STATEMENT

The following audit was performed on this study:

	CALENDAR
Audit of the Final Report	April 03, 2007

Audit report of the study report was transmitted to the Study Director for approval.

I certify that results presented in this report were generated using the materials and methods mentioned and that these results accurately reflect the Raw Data.

April 03, 2007

Nadine Pasquier

Quality Unit

Appendix 4

Protocol for binding assay 2 for Example 67 and Merck Example 3 enantiomers 1 and 2

Materials

- 96 well microtiter plates (V-bottom, polypropylene)
- Skatron cell harvester
- 37°C incubator
- Centrifuge
- Whatman GF/B Brandell cell Harvester Filters
- Cells expressing Dopamine D3 receptor
- [^{3}H]-DPAT (0.4 nM)
- Buffer A: (incubation) 50 mM TRIS (2-amino-2-hydroxymethyl-1,3-propanediol), 120 mM NaCl, 5 mM KCl, 2mM $CaCl_2$, 5mM $MgCl_2$, pH 7.4 @ 25°C
- Buffer B: (wash) 50 mM TRIS, pH 7.4 @ 25°C

Methods

For CHO D3 cells, media was removed and cells lifted from a flask with 5mM ethylendiamine-tetraacetic acid pH 7.4. All subsequent operations were performed at 4°C. Cells were pelletized by centrifugation at 1000 rpm or less for 5 minutes and the supernatant removed. Cells were homogenized 2 times with Polytron (20 seconds, setting 6) in 20 ml 50 mM TRIS, 5mM $MgSO_4$ at pH 7.4. The homogenate was centrifuged after each homogenization at 20,000 rpm at 4°C for 10 minutes. The pellet can be frozen at -70°C for approximately 6 months. The pellet was resuspended in a final volume of buffer A so that the concentration of tissue was 2.0 mg/ml.

Binding assay

Incubation mixture (volumes in µl)	
Ligand	25
Tissue	200
Drug or vehicle	25
Total volume	250

The reaction was started by the addition of tissue and incubated for 15 minutes at 37°C. The reaction was stopped by rapid filtration through GF/B (the filters were previously soaked in 0.5% PEI (polyethyleneimine) for 2 hours and dried). Filters were washed with ice cold buffer B in the Skatron harvester. Filters were dried overnight and counted in the Beta counter using Betaplate Scint.

Interpretation

Data are expressed as IC_{50} (the concentration that inhibits 50% of the specific binding) or as an apparent K_i, calculated by the formula:

$$K_i = IC_{50}/(1+[L]/K_d)$$

wherein [L] is the ligand concentration and K_d is the affinity constant for [^{3}H]-ligand, determined in a separate experiment.

Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

Appendix 5

Protocol for functional assay for Example 67 and Merck Example 3 enantiomers 1 and 2

Description of the cell line

The HEK293-G alpha15.D3 cell line stably expresses G alpha 15 which was generated by transfection of a HEK293 cell line with a G alpha15-blasticidin plasmid. The D3 receptor expression is maintained in the presence of puromycin. This cell line attaches poorly to typical tissue culture treated flasks. For a strongly adherent phenotype, the cells are grown on Matrigel (Becton Dickinson), diluted 1:200 with serum-free DMEM (Dulbecco's Modified Eagle's Medium) coated flasks.

Methods

20,000 cells were plated at 50 µl/well in a 384-well poly-D-lysine coated plate and the plates returned to a 37°C incubator overnight. 24 hours later, the growth media was removed and replaced with serum-free media in the presence of the calcium-sensitive fluorescent dye, fluo-4 (4 mM) and the active transport inhibitor probenecid (2.5 mM). The plate was incubated for 1 hour at 37°C in an incubator.

The media was aspirated and the plates washed with buffer 3 times with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffered saline (10 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KCl, 10 mM glucose, 145 mM NaCl plus 2.5 mM probenecid) to remove excess dye.

The plates were incubated for 15-45 minutes (in 30 µl) in an incubator at 37°C and the drug plate pre-heated for 15 minutes in the 37C incubator. The assay was set up in the FLIPR (Fluorescent Imaging Plate Reader) and fluorescence levels monitored continuously over a 90 second period.

Agonist/antagonist additions (15µl volume) were made simultaneously to all 384 wells after 20 seconds of baseline recording.

Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

Appendix 6

Analytical HPLC conditions for identification of Merck compounds

The compounds were separated on a Chiralcel OD-H column (250*4.6mm id); mobile phase heptane:2-propanol:diethylamine (80:20:0.1), flow rate 1ml/min, detection 225nm., at ambient temperature.

Enantiomer 1 eluted first (retention time: 7.646 minutes), followed by
Enantiomer 2 (retention time: 12.790 minutes).

Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

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Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479

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Andrew Martin Johnson B.A.
NOTARY PUBLIC
29 St George's Place
Canterbury
CT1 1UT, England
Tel: 01227 479479